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BIOMEDICAL AND BEHAVIORAL SCIENCES  
No. 31

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## AGROTECHNOLOGY

### AGRICULTURE ARRIVES AT A NEW STAGE

Moscow TEKHNICA I NAUKA in Russian No 11, 1977 pp 31-34

[Article by Academician N. Aleksandrov, All-Union Academy of Agriculture imeni V. I. Lenin]

[Text] Truly tremendous socioeconomic changes in rural areas have occurred in our country in the years of Soviet power. Socialist transformation of rural areas was one of the most difficult tasks that the Communist Party had to perform after the Great October Revolution. But the process of development is continuous.

In the decree of the Central Committee CPSU "On further development of specialization and concentration of agricultural production on the basis of interfarm cooperation and agro-industrial integration," there is indication of the routes to follow for solving the most important problems related to the new stage of implementation of Lenin's cooperative plan under conditions of well-developed socialism. Broad plans have been outlined for creating kolkhoz, sovkhoz and state-kolkhoz enterprises and associations in our country. Academician N. Aleksandrov of the All-Union Academy of Agriculture imeni V. I. Lenin tells us about them.

One of the chief distinctions of the present stage of development of agriculture is its intensification. If we compare the results obtained under the 9th and 7th Five-Year Plans, we shall find that the increase in grain, sugar beet, potato and sunflower production was due solely to increased harvests. (From 1965 to 1975, there was only a 4.1% increase in overall land area used for cultivation of crops.) Of the increase in production of vegetables and raw cotton, 70% is attributable to increased harvest and 30%, to increased planting; kolkhozes and sovkhozes began to deliver more milk primarily due to increased productivity, which contributed over 66% of the increment and the rest, by an increase in number of head of dairy cattle. The increase in production of beef and pork was achieved as a result of both productivity and increase in number of animals (in equal parts). The

chart illustrates the overall growth in production of most of the important forms of agricultural goods in the last 15 years. The annual mean gross product, as compared to 1965 figures, increased from 63.3 billion rubles to 90.9 billion rubles.

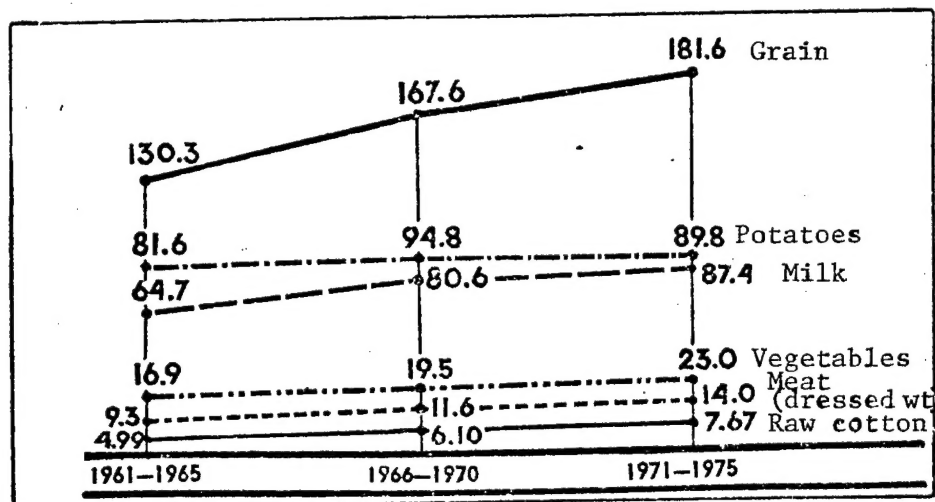


Operator Nina Mamonia  
at the central console  
for dispensing feed

The projected capability of the Donskoy complex in Stavropol'skiy Kray is 4,500 tons of meat per year. Young livestock is sent to the complex from farms in Trunovskiy, Krasnogvardeyskiy and Grachevskiy rayons of Stavropol'. Here, the calves present an almost 12-fold weight gain in 392 days.

There are only 50 operators who service the herd of 10,000 animals. They perform all of the laborious work by means of various mechanical equipment: self-propelled carts, transporters and dispensers. Experienced veterinarians supervise the condition of the animals. In forming the groups, a complete course of preventive inoculations

which also include antistress agents, is administered. There are 18 calves in each group and they are kept together to the end of the fattening period. Specialists believe that an unchanging, familiar environment is quite important to weight gain in young livestock.

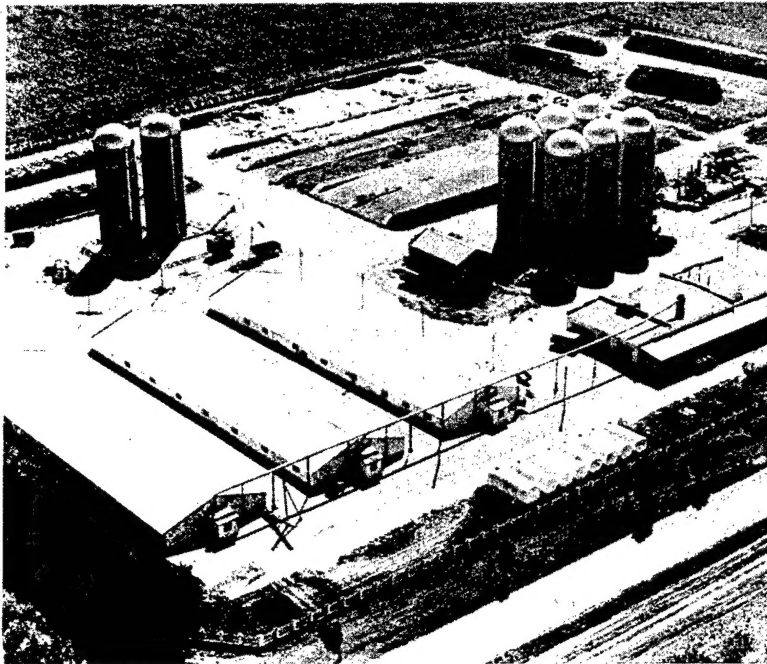


Dynamics of output of the most important forms of agricultural products in 1961-1975 (in millions of tons)

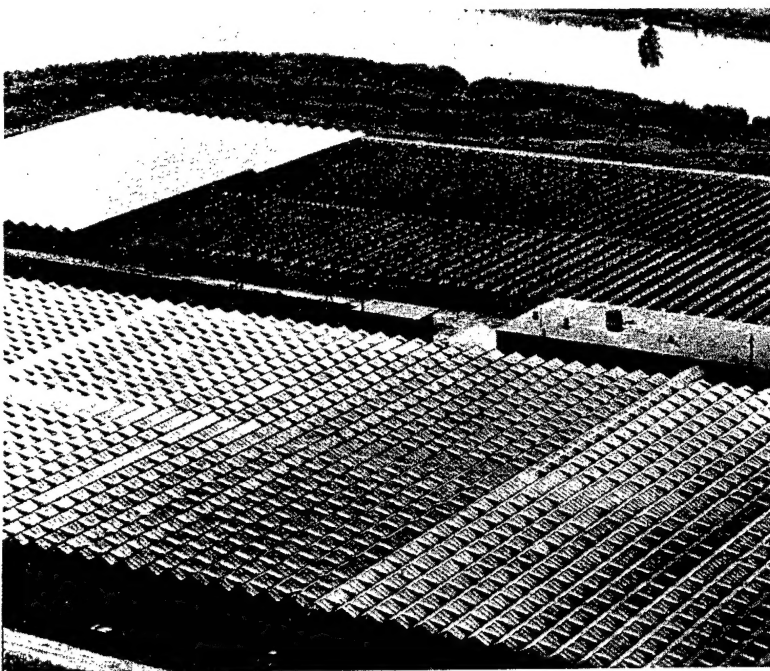
The share of agricultural workers dropped from 31% to 23% of all those employed in the national economy, in the period from 1965 to 1975. In the future, the figure will drop even more. Expansion of land area used will remain negligible. The conclusion derived from all the foregoing is unequivocal: the principal means of augmenting agricultural production is intensification thereof. This route is justifiably recognized by the Party as being the general direction, the main road of development of agriculture.

Intensification demands concentration and specialization of production; without this it is ineffective. The present stage differs from the preceding one in that these processes are best implemented mainly on the basis of intrabranh, interfarm cooperation and interbranch agro-industrial integration, rather than on the level of individual farms (although this is not rejected). What then are the new forms and structures in agriculture?

The simplest, primary form of interfarm cooperation is the development of interfarm enterprises. For this purpose, the funds of kolkhoz and sovkhos groups are combined, as well as part of their manpower and material resources. Otherwise, they retain completely their administrative and legal independence. Thousands of interfarm enterprises and organizations have been created in our country, and virtually all kolkhozes and a significant part of the sovkhos are involved in them.



Feed-producing  
kolkhoz complex



Hothouse complex  
in Kuban'

Thanks to such cooperation, it became possible to improve the work of all agricultural enterprises, rather than individual ones, as well as to standardize working conditions at farms varying in economic level and to upgrade the management system.

Agricultural production associations constitute a higher and more complex form of cooperation. They are characterized by common planning (all of the assignments on the state plan are submitted to an association as a whole), some centralization of management and resources (by means of establishing common funds and systems for rational redistribution thereof). The production and technical services are also unified (supply, marketing, warehouses, etc.). The integral mechanism of cost-accounting relations provides for common interests of all participants.

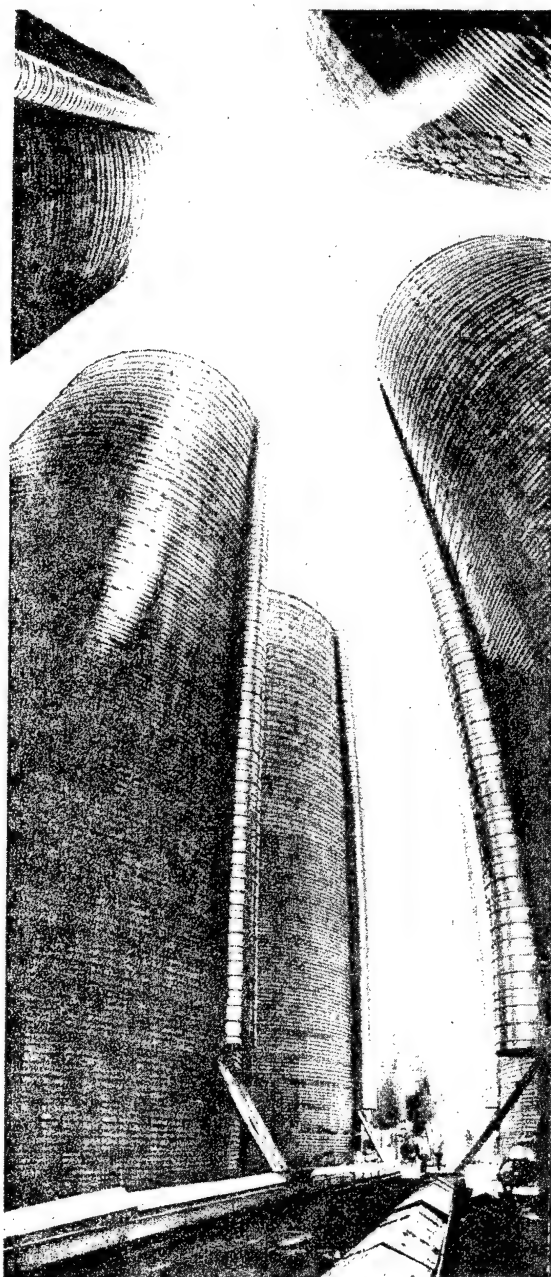
This form of cooperation is inherent in sovkhoses, and in some rayons, for example the Moldavian SSR, in kolkhoses as well. In the last few years, kolkhoses have joined intersovkhos associations on a contractual basis. In such cases, they usually produce different types of products. This is a transitional form, between interfarm enterprises and interfarm associations.

In addition to the above-mentioned forms of cooperation, others have now appeared: not only sovkhoses and kolkhoses, but scientific institutions, processing and marketing enterprises and organizations are broadly involved in associations. Agroindustrial enterprises and associations, scientific-industrial and agrarian-industrial-trade associations are being formed. The latter implement not only production, but delivery thereof to the consumer.

On the whole, of course, the national economy gains from such cooperation. But it is imperative to take into consideration the fact that its different elements--sovkhoses, kolkhoses, processing and trade enterprises and organizations--operate on different legal footings, their budgets, credit and profitability are different. Continued development of this form of cooperation will be quite difficult without standardizing all these characteristics.

And one should also not overlook the fact that sovkhoses and kolkhoses are agricultural enterprises having different forms of assets. Hence, there are also some differences in management methods, in relations with other enterprises, financial credit system and the government. At the present time, these differences are not manifested very strongly, since kolkhoses and sovkhoses are involved together mainly in creating interfarm enterprises and the most elementary transitional forms of interfarm and agro-industrial associations. But we must think about the future; eventually kolkhoz-state associations should become the most common form.

Participation of kolkhoses in interfarm cooperation and agrarian-industrial integration is also important for the following reasons: it aids in raising the level of socialization of kolkhoz production, improving public relations, bringing two forms of socialistic property and two types of agricultural production closer together and, consequently, gradually eliminating the



Silos of a new design

substantial differences between urban and rural areas. In this regard, it is an important and urgent task to perfect legislation, on which sovkhoz and kolkhoz work is based.

Broadening and deepening of agriculture's ties with industry and the economy led to development of a national economic agrarian-industrial complex. This is an intricate industrial structure, in which about 50% of our workers in the area of physical production are employed. Branches of this complex are undergoing more and more differentiation, as well as establishment for the first time. Reclamation, construction, repairs, the mixed feed industry, procurement--all this constitutes already an entire group of branches, the work of which is directed toward joint production of agricultural products. The systems approach, integration of all decisions, implementation of internal proportionality and balanced production are required.

Comrade L. I. Brezhnev indicated, in his report to the 25th Congress of the CPSU, with reference to the necessity of organizing and administering agricultural production: "Evidently, the time is coming to upgrade, and appreciably at that, production and economic collaboration between agriculture and the branches of industry with which it is directly related, to implement a standard national approach to development of the entire agro-industrial complex."

What then is the gist of this standardized approach? Let us discuss some of the problems, on the solution of which implementation thereof depends in many respects.



In our country, a sector-related system of economy management was formed. Interfarm enterprises and associations are also being founded on the basis of community of sectors. And this is understandable; cooperation among enterprises implies concentration of production on the basis of specialization thereof, existence of deep production relations, without which cooperation would become simply ineffective. However, agriculture differs from many other sectors in that it is "tied down" to specific territories and a territorial management system. For this reason, we cannot do without an optimum combination of the sectorial and territorial management principles. It should be noted that the question of combining sectorial and territorial aspects in planning and organization of agricultural production and management thereof is the most difficult and unexplored problem. In this regard, scientific institutions will have to make profound generalizations and analysis of the gained experience.

Development of the system of management of an agrarian-industrial complex is also linked with continued improvement of intersectorial economic relations. We refer to the fact that each sector of the complex must be concerned with development of dependent [or interrelated] sectors, as well as (and this is mandatory!) with obtaining the end product. In other words, achievement of maximum effectiveness in each sector should not occur at the expense of deterioration of economic indices of dependent sectors.

At the present time, this is not implemented completely. Moreover, stimulation of many servicing sectors is, in a number of cases, in direct opposition to the interests of their principal consumer, agriculture. For the criteria for evaluating their activities should aid in obtaining an end product of better quality and at lower cost, rather than greater profit, as is the case at the present time.

Let us consider, for example, Soyuzsel'khoztekhnika [All-Union Agricultural and Technical Association]. This is a large organization that does very much work for agricultural enterprises. Their material and technical resources constituted almost 20 billion rubles per year, and they are growing annually. This association performs a large volume of annual work, being concerned with industrial servicing of agricultural enterprises, in excess of 8 billion rubles. This includes equipment repair, freight hauling, installation of equipment at livestock farms, performance of a number of specialized agricultural tasks, etc.

Gradually, the operation of this system is improving; however, the consumers of its services, kolkhozes and sovkhoses, are far from satisfied with their quality, delivery time and cost. The monopolistic status of Soyuzsel'khoztekhnika in performing major repairs, supplying spare parts and scarce equipment, and providing automotive transport does not always suit the kolkhozes and sovkhoses. For this reason, it is logical for them to try to organize an industrial service within the boundaries of their farm or on an interfarm basis. Quite often, this is not better or cheaper than with Sel'khoztekhnika, but it is more satisfying to the farms, since it enables them to juggle the resources.



Автоматизированный свиноводческий комплекс.

Automated swine-breeding complex

Then, perhaps, it would be better to unite Sel'khoztekhnika with, for example, the Ministry of Agriculture? No, an uncontrollable system could be produced if these problems are resolved by merging several ministries and agencies into a single body, which appeared to be the simplest, but is practically impossible. A much more effective solution is, specifically, cooperation. Thus, the short experience gained in creating an industrial service on an interfarm basis, with the participation of Sel'khoztekhnika enterprises, is indicative of encouraging results. Perhaps, such cooperation will turn out to be promising, especially if evaluation of, for example, tractor and machinery repair by Sel'khoztekhnika will be made on the basis of a previously determined coefficient of their technical readiness, while evaluation of the quality of installation work in livestock premises would be based on continuous operation of equipment, etc.

Similarly, it would apparently be also desirable to create, on an interfarm basis, associations in which kolkhozes, sovkhoses and enterprises of the mixed feed industry will participate. This would eliminate the flaws that still exist in supplying agricultural enterprises with mixed feed. These flaws are attributable to the low capabilities of some plants, shortage of some ingredients, for which reason a large amount of grain is unreasonably utilized (see "Bacteria at Work," published in this journal, No 2, 1977--editor). Moreover, the mixed feed industry is often remote from the consumer area and consumers have no way of influencing producers.

In general, it should be stated that agriculture presently consumes an enormous amount of physical resources and industrial services that were created in other sectors of the national economy. Five-sixths of its fixed production capital are formed at the expense of the production of other sectors. In current expenditures, there is a high share of former, reified labor, and it is constantly increasing. For example, physical input [outlay] constitutes about 60% of the cost of agricultural kolkhoz production and even 62% in the livestock industry (1975).

The rapid growth of productive capital and slower growth of gross production lead to an increase in its capital-output ratio, i.e., an increasing amount of prior labor referable to tractors, machinery, buildings, fertilizers, mixed feed, etc., is required for the production of each unit of agricultural product. K. Marx wrote that the labor that it costs to produce machinery should be less than the labor that is replaced by the use thereof. In other words, new technology should always yield an effect. If there is none, such technology does not cause an increase in productivity of labor. In this case, the capital-output ratio and production cost must rise. At the present time, the technology producer often gains a greater effect than its consumer, and this is absolutely abnormal.

This is why the cost of some types of agricultural products is rising for the last few years. For example, as compared to 1971, the cost of sunflower seeds in kolkhozes rose by 15% in 1975. Of course, this is partially attributable to the substantial increase in kolkhoz and sovkhos worker wages. But the main cause is increase of the capital-output ratio, which is related to higher prices for production tools and production services that are delivered to agriculture from its partners in the agrarian-industrial complex.



In Rostovskaya Oblast, a new industrial livestock complex, Proletarskiy, has been put in operation; it has a capacity of 20,000 head of cattle. A total of 63 farms regularly deliver young livestock to this complex. There are 26 people directly involved in fattening the animals

Generally speaking, questions of pricing in agriculture proper and industrial sectors of the agrarian-industrial complex require in-depth work.

Lowering the capital-output ratio, the physical output ratio, is an important prerequisite for continued increase in effectiveness of agriculture. This applies the most to the livestock industry and particularly feed. Some interesting data were obtained at the Yelanskiy Pedigreed Stock Farm in Voronezhskaya Oblast. They revealed that the more feed was consumed per cow, the lower the milk cost. Thus, in a group where the cows were better fed, the milk yield was almost 2.8 times higher than in another group, with poorer feeding; the feed outlay per kilogram milk was almost 50% less and its cost 40% less. Hence, it can be concluded that one must make a decision that appears paradoxical at first glance: to increase the amount of feed per head of livestock and, in some cases, to use even more expensive feed, in order to lower cost thereof per unit product.

All these are elements of an important economic problem, that of systematic intensification of the livestock industry, as well as all agriculture. Experience, which is already vast, constantly confirms the fact that this can be achieved, as we have noted, by means of concentration and specialization of production. But one must bear in mind the words of V. I. Lenin, indicating that the effectiveness of concentration has no absolute significance, but is determined by the status and short-range prospects of technological progress, implementation of which, in turn, is prompted and expedited expressly by concentration and specialization of production.

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## AGROTECHNOLOGY

### THE BIOSPHERE--REALM OF REASON

Moscow TEKHNICA I NAUKA in Russian No 11, 1977 pp 35-37

[Article by Academician A. Sidorenko, vice president of the USSR Academy of Sciences]

[Text] The Communist Party and Soviet government display constant concern about protection of the biosphere, as man's habitat and one of the main sources of wealth and welfare of society. The 25th CPSU Congress stressed, with new forcefulness, the increasing importance of environmental protection. In the proceedings of the Congress, this problem is viewed as an objectively valid phenomenon and, at the same time, as the subject of constant concern and deliberate activity of the Party and state. Our journal has also devoted much attention to this topic (see articles under the heading of "Industry and the Environment," Nos 4, 6, 7, 8, 10, 1977).

This article by Academician A. Sidorenko, vice president of the USSR Academy of Sciences, deals with various aspects of interaction between the environment and society.

The biosphere is the envelope around earth, within which reasonable human endeavor is now becoming the main and decisive factor of development. Let us quote a few figures to vividly illustrate the scope of such endeavors.

Each year over 4 billion tons of petroleum and natural gas, more than 2 billion tons of coal, almost 20 billion tons of minerals in the form of ore and rock are obtained in the world. Fuels, ore and rock penetrate to the earth's surface, in the air, soil and water, when they are refined.

In the 1970's, about 2 million various chemical compounds, not counting chemical fertilizers, were released into earth's biosphere. According to the estimate of WHO, mankind uses about 0.5 million chemicals. There are data on the deleterious properties to man of 40,000 substances, including 12,000 toxic ones used in industry, which sometimes penetrate into foods. Each year, more than 250,000 new chemical compounds are synthesized in the

world, and about 300 of them are used in industry and could be released into the environment.

All this is causing some alarm in society for its future, for purity of water, soil and air, for the condition of the natural environment. The problem of the environment has become just as vital as supplying man with food or energy.

However, this problem should not be reduced solely to the negative aspects of man's influence on the environment. The adverse consequences cannot be compared to the enormous general effect obtained by virtue of harnessing the forces of nature, which enabled man to improve sharply his living conditions, i.e., to create a new, "man-made" environment. One does not have to reach far for examples.

It was possible to create an utterly new environment in a number of regions by delivering water to deserts and drying swamps. Malaria, which used to take thousands of lives, has been eradicated from the humid subtropics. The energy resources have made it possible to radically alter man's micro-environment. Let us consider, for example, life in the arctic region. A nice and warm apartment, hot water, modern work premises, municipal transportation in the northern regions constitute the artificial environment, in which man spends most of his time. It is just as important to him as the virginal beauty of his surroundings. We have spoken much about destroying forests, but do not stress the fact that urbanization is almost always associated with development of forests of the park type, and that man-made forests are becoming commensurate in size with natural forests.

Under the present conditions of intensive development of technology, man as a producer and consumer is coming more and more often in direct contact with the new "artificial" environment and its components, with a new, technogenic biosphere, a biotechnosphere. Progress of human society requires development of industry, and the technophobia that permeates many articles dealing with environmental protection (as if it was the environment that needed protection, rather than we) not infrequently turns an indifferent ear to the fate of people.

Society, which eliminated the destructive effects on the biosphere, will have to transform it into a biotechnosphere by means of reasonable and purposeful activity. This transformation, to be made on a scientific basis, will be directed toward progressive development of the habitat. In other words, man must design and develop the natural and technical environment he requires to exist. The reality of such control is determined not only by the capabilities of science, but primarily by appearance of the socialist regime and its increasing influence on the fate of the world.

In the USSR, environmental protection has been promoted to the rank of national policy. The Central Committee of the CPSU and USSR Council of Ministers are not sparing with regard to funding environmental protection.

The plans call for spending 3 billion rubles (in the most important sectors of industry) on protection of the air basin against pollution by stationary sources under the 10th Five-Year Plan. In the same years, there will be major construction of treatment plants in cities, rural areas and industrial enterprises. The overall capacity of these installations will be more than 70 million cubic meters per day.

There are plans for measures to preserve the fishery-related importance of our reservoirs, particularly the southern seas. This applies in particular to the Caspian and Azov seas. A total of 50 million rubles will be allocated directly for work dealing with preservation and restoration of fish resources.

Much will be done in agriculture. Agrotechnical measures will lead to additional retention of 25-40% of surface drainage and lower soil erosion by an average of 50%; forest-reclamation work will reduce moisture loss by 30-50%. There are plans to develop and introduce on a broader scale biological agents for the control of diseases and pests of agricultural crops.

Intensive cutting of forests elicited justifiable alarm. At the present time, the situation is being remedied. In the past few years, there has been an increase in share of deep processing of wood pulp, which diminishes the amount of waste in this industry. There has been an increase in volume of reforestation work. Under the 9th Five-Year Plan, reforestation and afforestation were performed over an area of 12.1 million hectares. Under the 10th Five-Year Plan, the measures to ameliorate forests will be implemented over an area of about 13-14 million hectares.

At the present time, there are 104 preserves, 600 national sanctuaries over an area of about 15 million hectares and about 1000 local sanctuaries.

Planning of measures to protect the environment is based on technological policy, which provides not only for eradication of the consequences, but primarily elimination of the very causes of environmental pollution. The first and foremost task is to assimilate waste-free technology with closed systems of water and air circulation, etc. (more details on this score are contained in the article, "We Should Learn From Nature," in this journal, No 8, 1977--editor). But it should be noted that a turn to such technology involves major expenditures for one time only. For example, heretofore an average of about 20 million rubles of capital investment was required to set up every cubic kilometer of water in a recycled system.

Since 1975, the capital investment for implementation of combined and rational use of natural resources and environmental protection is allocated in a special section of the national economy plans. The outlined measures for environmental protection are now a component of long-range 5-year and 1-year plans of development of the national economy.

In all, 11 billion rubles have been allocated under the 10th Five-Year Plan for environmental protection, which is comparable to the annual budget of a



number of large ministries. Significant allocations were also made in prior Five-Year Plans.

Aside from growth of special, target-oriented capital investments, there is also growth of the share of one-time expenditures to prevent environmental pollution. Allocation of these funds is made in drafts and estimates of enterprises under construction in all sectors of the national economy. Thus, in the plans of modern industrial enterprises, 5-20% of the total capital investment is to be spent on environmental protection. There is also more rigid control of dumping deleterious substances into the environment (see an article on this subject in this journal, entitled "There Will Be No Smog," No 10, 1976--editor).

In the USSR, maximum permissible concentrations and other standards have been set for over 2500 substances dumped in the environment, in particular for the air of work areas in industrial premises, atmospheric air of urban regions, water in reservoirs, soil and foods. Let us mention that, in the vast majority of cases, the Soviet standards for quality of the environment are more rigid than in foreign countries.

With reference to the environment, we should first have a clear idea what it consists of. In our country, particularly in the popular science literature, newspaper articles, on radio and television, the concept of the natural environment around us is reduced mainly to the atmosphere, water basins, soil, the animal and plant kingdoms. Without minimizing by any means the importance of these elements of nature, I should like to call attention to the lithosphere, the crust of the earth, on which the animal and plant kingdoms develop and with which the water and air basins are inseparably linked.

Earth's crust is one of the most important environments of man, the main and decisive element of the biosphere. One should not overlook the fact that, because of development of the mining industry and inevitable removal of considerable areas of land to mine for raw mineral and fuel material, construction and location of solid industrial waste, we are faced with the serious problem of reclaiming and restoring the productivity of land impaired by industry. We are delving deeper and deeper into the earth, and it is often more difficult to eliminate the consequences of our intervention than pollution of water or air. There must be intensification of research on the effects of such phenomena and protection of earth's crust as the environment in which man lives and works (work that is being done in this direction is discussed in the article, "Protecting the Earth," in this journal, No 10, 1977--editor).

In general, it must be stated that technological progress and intensification of anthropogenic effects on the environment disclosed the paucity of our knowledge about the main properties of the environment that surrounds us, is customary and, it would appear, had been well-studied, as well as forms and levels of its organization and structural mechanisms of its

self-regulation. Our limited knowledge was also revealed with regard to the results of affecting the environment, lack of methods of predicting them and lack of understanding of the mechanism of occurrence of socioeconomic contradictions in making use of natural resources. As a result, implementation of major national economic plans (for hydraulic installation construction, reclamation, use of chemistry in agriculture), which are directed toward achieving a higher technical and economic effect, often causes unforeseen adverse consequences in the environment, lowering the social and economic effect and requiring new capital investment.

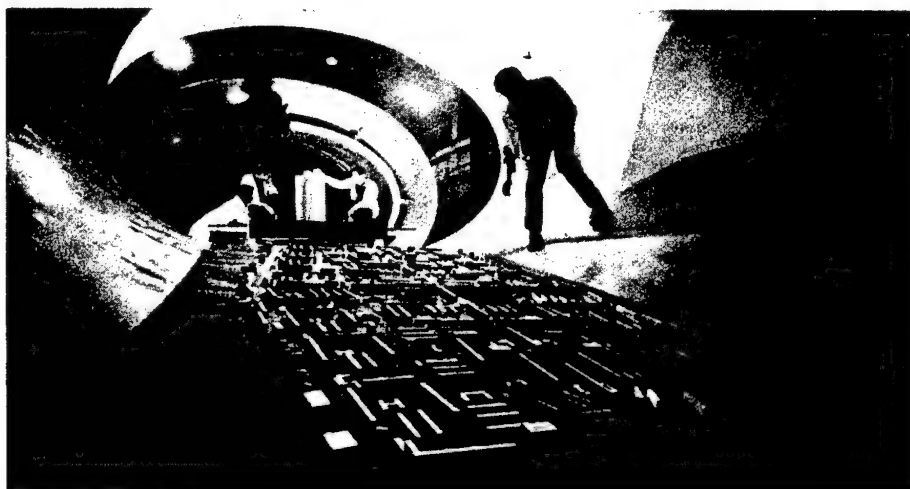
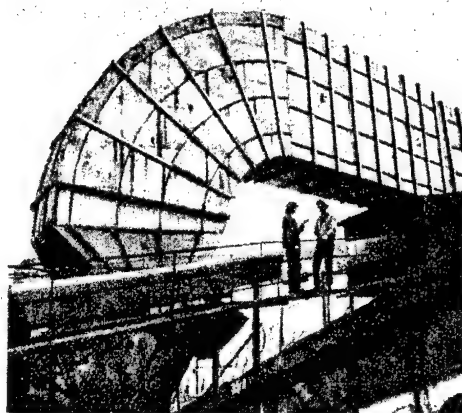
We see that practical implementation of "control of the environment" is becoming a reality. This is why it is imperative to investigate the natural patterns of development of the biosphere, to analyze the reactions of its components to alien factors on different scales and determine the range of reversibility of changes in the biosphere, to forecast the reactions to different variants of factors and evaluate them (including quantitative assessments), both from the standpoint of natural history and usefulness or detriment to society.

Information about balances of matter, energy and ecosystems is required to solve these problems.

We must stress the complex, multifaceted and interdisciplinary nature of these basic studies of the natural and, particularly, technogenic environment. For this reason, representatives of different sciences must participate in the development of the socialistic conception of protection and rational use and reproduction of the environment.

Problems of environmental protection and utilization of natural resources are not solved in the same way under different socioeconomic systems. In a capitalistic society, in the presence of bitter competition, the use of natural resources is in the form of predatory exploitation. There, the relative alleviation of ecological problems is related to the restrictions imposed by government regulations on haphazard capitalistic production and initiation of national measures to protect the environment. The monopolies try to pass on a significant part of the expenses related to environmental protection to the workers. Only a socialist system of national economy, that serves the interests of all people and creates objective prerequisites for a basically new, harmonious unity of the industrial society of people and nature, is capable of providing a cardinal solution to these problems.

It must be borne in mind that work directed toward environmental protection and amelioration of the biosphere cannot be limited to activity on a national scale. Environmental pollution, which appears on the territory of one nation, shifts to other countries. There are many natural factors involved here: riverways, air flow, marine currents, etc. For this reason, joint and coordinated measures must be implemented by many countries. Thus, it is very obvious that the question is raised of broad international collaboration, both scientific and social.



High-power units have been installed for the three smelter furnaces at the Zestafoni Ferroalloy Plant (Georgian SSR) to treat industrial waste containing metal dust and gases. In one hour, about 600,000 m<sup>3</sup> air passes through them. It is discharged into the atmosphere in completely pure form, while the "trapped" elements, up to 22 tons of metal dust per day, are returned to the furnaces for resmelting.

Top photograph: main intake ventilation duct.

Bottom: Clean air for cities of the future. The results of testing the models of new projects in a wind tunnel will tell how best to locate buildings and blocks.

We should stress the particular importance of international detente, change in the system of international relations on the basis of the principle of peaceful coexistence of nations with different socioeconomic systems to the solution of problems of environmental protection. Peace and international

collaboration will create the most beneficial climate for successfully solving the entire set of ecological problems.

In this regard, the decisions adopted by the Conference on Safety and Collaboration in Europe, which convened in Helsinki, played a basically important role.

At the present stage of development of society, when the ecological role of man has assumed planetary dimensions, when man has become the main moving force in the biosphere, it has advanced, in the definition of Academician V. I. Vernadskiy, to a new and higher level, the noosphere, or sphere of of reason.

How we are to manage on earth to better satisfy the needs of society with respect to natural resources, to comprehensively replenish them with the least detriment to nature--the hard crust of the earth, the hydrosphere and atmosphere with the diversity of living beings in them--depends on the wisdom of society, on all of us. We should also think about how we shall build the new biosphere, which we shall leave to future generations.

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## AGROTECHNOLOGY

### INDUSTRY AND THE ENVIRONMENT: CEMA AND ENVIRONMENTAL PROTECTION

Moscow TEKHNICA I NAUKA in Russian No 11, 1977 p 37

[Article by Yu. Belichenko, member of the Council for Protection and Amelioration of the Environment, CEMA Committee for Scientific and Technological Collaboration, vice chairman of the Commission for Protection of Water Resources, Central Administration of the Scientific and Technical Society of Agriculture]

[Text] Scientists, engineers and designers of socialist countries are searching for basically new roads leading to cardinal solutions to problems of protection of the biosphere from the deleterious effects of man's industrious endeavors.

The complex program of socialist economic integration provides for joint work on major problems in the area of rational use and protection of natural resources. For this purpose, in 1972, the CEMA Committee for Scientific and Technological Collaboration established the Council for Protection and Amelioration of the Environment, which is a permanent body of the former.

The council coordinates work conducted in CEMA agencies (as well as along other channels of multilateral collaboration), development of proposals related to cooperation and specialization of production of instruments, equipment and measuring techniques to protect soil, water and air basins from pollution, as well as to prepare recommendations dealing with effective forms of collaboration in the area of environmental protection.

In 1974, this council prepared the "General comprehensive program for collaboration of CEMA member nations and SFRYu [Socialist Federated Republic of Yugoslavia] for the period up to 1980, in the area of protection and amelioration of the environment and related rational use of natural resources." This program includes 11 problems (155 topics) of utmost importance, including problems of a socioeconomic and organizational-legal nature.

Collaboration between CEMA member nations in this field is developing well and yielding good results. Socialist nations are concentrating on individual scientific-technical and technico-economic problems of theoretical and practical interest on the basis of international sharing of work; they are

saving funds and time and eliminating unjustified duplication of research. Such collaboration is based on the knowhow and achievements accumulated in this field by all fraternal countries. As a result some important solutions have already been found.

In the period of 1972-1975, about 750 scientific research and design projects have been completed. They include 15 theoretical investigations, 123 projects dealing with development of new technological processes to reduce environmental pollution, 140 topics dealing with development of methods of conducting research, setting norms and standards for detection of pollutants; 141 projects dealt with development of apparatus, instruments, units, equipment, new filtering material for treatment plants and other technological items.

Of the above-mentioned completed projects, over 180 have been introduced to industry and are used in the form of methods, standardized methods and norms. We can mention, for example, the following specific projects.

For the last few years, CEMA member nations worked actively to upgrade methods of estimating all components of the water balance and particularly water drainage as one of the most important hydrological characteristics for planning and designing in the area of water management. It is now possible to use computers to calculate water drainage over a period of many years or less than a year, to predict the quantitative changes in surface water resources and quality thereof as a result of industrial activity. These studies were particularly important to areas with concentration of population, industrial and agricultural products of CEMA member nations, including most of the GDR, Polish People's Republic, CSSR and the European part of the USSR.

Proposals have been prepared on creating automatic control [inspection] stations for surface water quality and processing of the information obtained on computers, as well as introduction thereof in the practice of the water management system.

Use by CEMA member nations of the prepared "Standardized methods of testing water quality" makes it possible to obtain comparable results, reduces time required for analyses and increases their accuracy, which is very important to implementation of laboratory surveillance of the condition of surface, underground and sewer water.

According to estimates made in Soviet institutes, in our country the cash saving resulting from this recommendation, as well as "Methods of treating and processing liquid waste from diverse industries," constituted about 1 million rubles.

Let us quote other examples of the effectiveness of joint work: Introduction of a bag-type filter to remove dust from gases to the Volkhovskiy Aluminum Plant will save about 300,000 rubles per year. Use of the method of alkaline absorption with soda and milk of lime, which extends the

operating life of absorption systems, may save an average of about 900,000 rubles per year for an industrial enterprise.

Introduction of the method of removing zinc oxide from ventilation gases by means of cloth filters in the GDR, at the Mansfeld Metallurgical Combine, will save about 85,000 marks per year, while the use of methods of highly effective treatment of technological gases from shaft furnaces that remelt scrap metal at the A. Funk Mining and Smelting Combine resulted in a saving of 400,000 marks per year.

Organization of collaboration is also effective in working on plans for territorial industrial complexes, in which there are provisions for a common industrial cycle containing the technological cycles of different enterprises, which would rule out completely environmental pollution.

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CLASSIFICATION OF LAND AND ITS USE IN THE USSR LAND CADASTER SYSTEM

Moscow ZEMLEDELIYE in Russian No 3, 1978 pp 18-21

[Article by Yu. F. Fedorin, Director, Soil Analysis and Land Classification Division, State Scientific Research Institute of Land Resources, USSR Ministry of Agriculture]

[Text] Now that agricultural production is intensifying and becoming specialized, the most sensible and fullest use of all land resources is acquiring especially great significance. The USSR Council of Ministers decree "On the Order for Managing the USSR Land Cadaster" (10 June 1977) is aimed at these tasks.

The land cadaster is a system of data on the quantity and quality of land, its legal status, and its economic potential. The "Fundamental Principles of USSR and Union Republic Land Legislation" (Section VIII) clearly defines the content, components, and purpose of the USSR land cadaster.

In its broad sense, a land cadaster is defined as a register, a description, and an assessment of land in relation to its natural qualities and productivity; it also subdivides land into groups and classes.

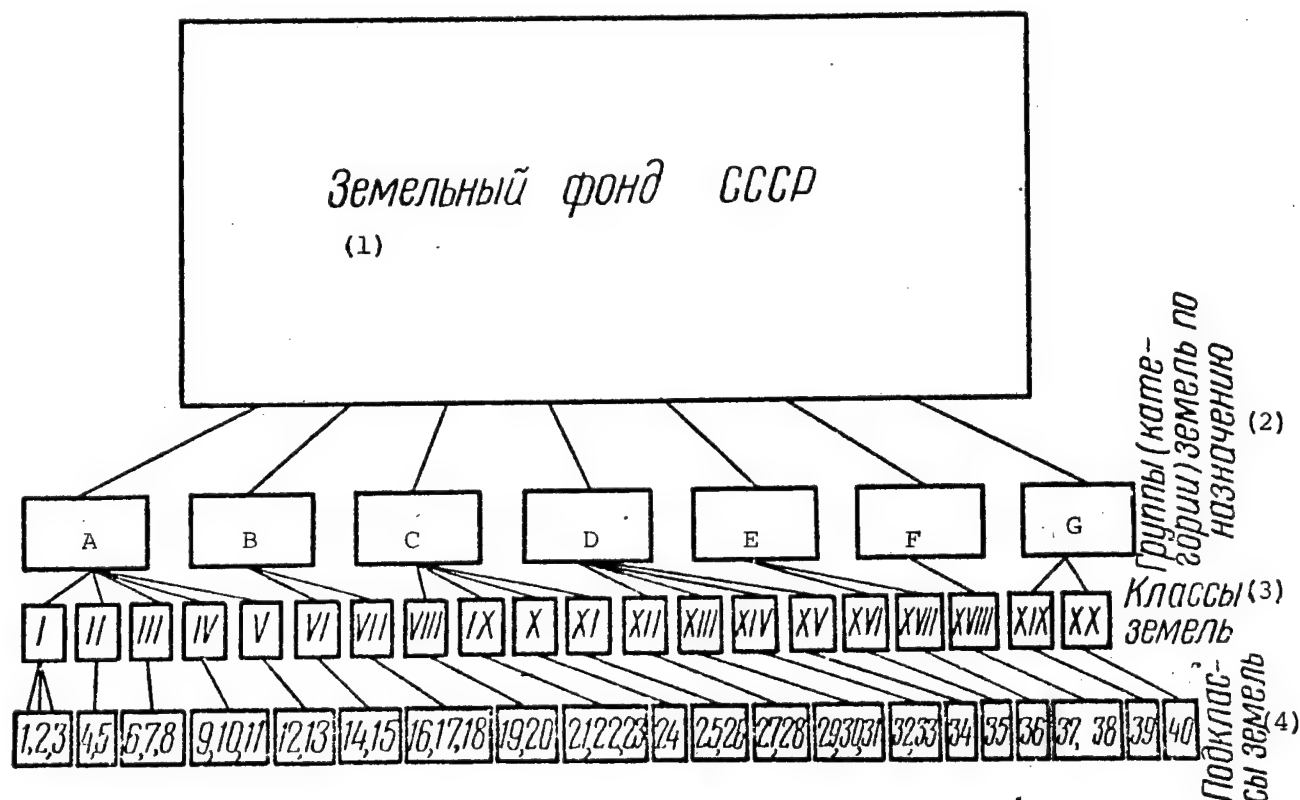
A tremendous diversity of soil and land, upon which the quality of agricultural land and the possibility and nature of its use depend in many ways, is encountered on the broad expanses of our country, from the cold tundra to the hot sandy deserts of the Turkmen ASSR and the moist subtropical regions of the western Georgian SSR. Consequently this land must be assessed in relation to so-called natural capacity ("land usefulness").

The land fund classification serves this purpose. This article discusses development of such a classification in our country.

The assessment of land took account of the ecological conditions of the zones in addition to soil features. These include climate, topography, vegetation, soil-forming rock, hydrogeological features, human economic activity, and other conditions upon which the methods for heightening fertility and improving land use depend.



The land classification describes the land and classifies it in relation to the country's natural-agricultural zones. Seven groups are distinguished in relation to natural capacity in all zones with developed agriculture: A. Tillable land; B. hay land; C. grazing land; D. land in the reclamation fund; E. land little suited to agricultural purposes; F. land unsuited to agricultural use; G. land disturbed by mining, construction, and so on (see figure).



Key:

- |  |                    |
|--|--------------------|
| 1. USSR land fund                            | 3. Land classes    |
| 2. Land groups (categories) based on purpose | 4. Land subclasses |

Depending on soil properties, topography, and other conditions, land in each group can be used differently. For example tillable land can be used for orchards, intensive cultivated pastures, and so on, hay or pasture land located in floodplains can be used to grow vegetables, and so on.

Land in the reclamation fund includes that requiring stubbing, rock removal, draining, irrigation, or other types of reclamation.

The seven groups of land listed above are in turn subdivided into 20 classes and 40 subclasses. The classes consist of land with similar natural and

economic properties used in the same way, and subjected to similar methods of cultivation and productivity improvement.

The land subclasses differ in relation to the procedures of land use and the methods for heightening soil fertility, which depend on the soil-forming rock, the mechanical structure of the soil, the degree of salinity and alkalinity, erosion, and other properties.

Five classes (from I to V) are distinguished in the tillable land group (A).

Class I contains cultivated land. It includes irrigated and drained land improved by prolonged application of fertilizers and utilization of sophisticated agricultural procedures to such an extent that this land differs fundamentally from similar contiguous parcels, chiefly in relation to its fertility. Given economic feasibility, the quality of such land permits its use in all agricultural sectors.

Class II tillable land includes drained (not eroded, not inundated) watersheds and slopes with a grade of up to  $2^{\circ}$  and with soils having similar production properties, overlying uniform bedrock. There are two subclasses in this class: 4--With loamy and light loamy noncalcareous soil, and 5--with loamy calcareous soils. Parcels that are mildly and moderately rocky can be distinguished in each subclass. This is essentially the best land in each zone after cultivated land.

There are three subclasses in class III tillable land. Parcels in subclass 6 have sandy loam and sandy soil overlying corresponding bedrock, and both mildly and moderately rocky land are included. Subclass 7 contains parcels of drained watersheds and slopes of up to  $2^{\circ}$  with loamy soil over rubble-pebble deposits or eluvial-deluvial bedrock. Here the bedrock noticeably worsens the economic merits of the tillable land.

Class IV in group A land contains poorly drained, periodically overmoistened lowland plains with weakly gleyey (this is in relation to well-moistened regions) and weakly salinized and alkaline soils and their complexes (in weakly and poorly moistened regions). There are three subclasses in this class. Subclass 9 contains parcels with clayey and loamy calcareous soils, subclass 10 contains land with noncalcareous soils, and subclass 11 consists of land with sandy loam and sandy soils formed on loam and clay. Moderately rocky parcels can be present in all of these subclasses. In regions of sufficient and excessive moisture, crops often become waterlogged, the irrigation schedule is disturbed, and maturation is retarded on land of this class due to overmoistening, while in steppe and arid regions the yield may decline noticeably on alkaline and even saline soils.

Class V tillable land encompasses sloped land. Here it is important to implement a complex of antierosion measures.

As we can see from this classification, the larger the serial number the relatively lower is the suitability of the land for agricultural production.

The economic feasibility of using each land parcel is accounted for when developing land use plans and compiling land maps.

Hay land (group B), which includes two classes (VI, VII) could be used not only for hayfields but also simultaneously for hayfields and pastures, while when necessary floodplain, irrigated, and drained land can be plowed as well.

The grazing land group (group C) contains four classes (VIII-XI). This group includes overmoistened and inundated land in zones of excessive and adequate moisture, alkaline and sandy soils in arid zones, and land on the steep slopes of gully-ravine systems, or mountain slopes with rocky or eroded soil, which due to topography and productivity cannot be used as plowed and hay land but is fully suited to animal grazing (especially sheep), all the more so because it covers extensive territory.

As had been noted above, land unsuited to agriculture as well as land which could not be used even for forestry without preparation is placed in special groups: D. Land in the reclamation fund; E. land little suited to agriculture; G. land disturbed by mining, construction, and so on.

Land in the reclamation fund (group D) is subdivided into four classes (XII-XV). These include both land in lowland and transitory marshes, peaty to varying extents and considerably gleyed (subclass 27), and mineralized land (subclass 28). This land requires drainage, after which it is usually transferred to the appropriate class or subclass in the tillable, hay, and grazing land groups.

Class XIII of this land group includes highly salinated soils (subclass 29), very highly salinated soils including solonchak (subclass 30), and desert soils (subclass 31). This land is widespread in the desert and semidesert regions of Central Asia and the Kazakh SSR. This land must be drained, excess salts must be leached out meticulously, and regular irrigation must be organized.

Class XIV of the group of land in the reclamation fund (group D) includes gulleys and ravines that must be planted with grasses (on the slopes) or trees. Such land exists everywhere, but it is especially widespread in steppe and forest-steppe zones, and it is represented by active erosion gulleys and the slopes and bottoms of ancient ravines, both on loose rock (subclass 32) and on dense rock (subclass 33). Class XV of this land group contains shifting unsodded sands--barhans, sand dunes, and others (subclass 39). Saxaul, tamarisk, oleaster, and other species are planted on such land to secure the sand and permit sheep grazing (when water sources are available). Shifting dunes in regions of sufficient moisture (the Baltic coast, and elsewhere) can be used for timber stands.

Land little suited to agriculture (group E) is represented by high marshes (class XVI, subclass 35) and poorly developed, usually rocky rock deposits (class XVII, subclass 36). Bare rock (cliffs, very steep mountain slopes, rocky talus, and so on) is little suited to agriculture.

Land totally unsuited to agricultural development (group F) has no soil cover. This land essentially includes glaciers, permanent snowpack in mountains, and parcels under water (class XVIII, subclass 37), as well as bare mountain cliffs (subclass 38).

The land classification also foresees land served by mining (group G). Such land includes exhausted peat bogs (class XIX, subclass 39), ore and nonmetal-liferous mineral quarries, rock dumps, and others (class XX, subclass 40). Without preliminary recultivation, agricultural and even forestry use of such parcels is impossible.

Although it is very expensive to restore such land, it is suited for use as timber stands, hotbeds, pastures, orchards, dairy farms, reservoirs, vacation zones, and so on.

The State Land Cadaster contains a section, based on the USSR land classification scheme, in which the qualitative state of land is described and registered; this permits sensible use of the land, establishment of the volume of land reclamation needed, and revelation of new land suitable for addition to agricultural turnover.

A land map is compiled on the basis of a production classification of land. In this case we use existing soil and topographical maps, and cartographical and published sources describing the given territory in relation to climatic features, superficial geological (especially Quaternary) deposits, and other conditions. It is only through such integrated consideration of ecology that we can draw a land map for any territory without significant errors.

Thus on the basis of soil, topographical, and other maps specialists draw a land map, a new document having not a narrow (soils, topography, vegetation, and so on) but rather an integrated, economically applied significance, especially to agriculture.

Experience has shown that land maps (compiled for real objects in various zones on an experimental basis) are simple to use, and that they account for all ecological conditions having the greatest influence on land productivity. Recommendations concerning the best use of the land in each class and subclass are indicated in map summaries. There is no need to provide a detailed description of the genesis of soils, bedrock, the nature of climate, topography, and so on here.

The proposed land classification has been approved by the Scientific-Technical Council of the USSR Ministry of Agriculture. It affords a possibility for scientifically grounding a proper ratio of agricultural and forest land, water sources, preserves, vacation zones, and other objects, as well as for improving land use and protection.

Use of the recommended land classification will make it possible to correctly locate agricultural land, develop sensible agricultural procedures, implement the necessary reclamation procedures, and so on. For example consideration of the quality of land in the desert-steppe zones of the USSR (17.4

million hectares) has established that out of the total area of tillable land (2.7 million hectares) only one third can be interpreted as being in the first class, while the rest of the land requires integrated development; more than 5 million hectares of grazing land in the tillable land group can be improved significantly.

A land map drawn on the basis of a survey of Starooskol'skiy Rayon, Belgorodskaya Oblast (the basin of the Kursk magnetic anomaly) made it possible to establish that over 80 percent of the land spoiled by mining is in classes II and III. More than 30,000 hectares of poorly productive tillable land have been revealed (land in the poorer land classes); this land could be improved by returning the fertile chernozem layer removed by mining operations. Proposals have been developed for heightening the productivity of other land.

The land classification and land maps contain valuable information permitting grounded transformation of land, its protection, and revelation of the reserves of productive land, information that could be used in natural-agricultural and other regionalization efforts, information with which to develop agricultural management systems, to determine the volumes and places of recultivation, and so on.

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PRESENT-DAY STATE AND PROSPECTS OF INVESTIGATION OF CARDIAC RHYTHM DISTURBANCES

Moscow KARDIOLOGIYA in Russian No 4, 1978 pp 5-11

[Editorial by N. A. Mazur, All-Union Cardiological Scientific Center of USSR Academy of Medical Sciences]

[Text] In the last decade for the investigation of cardiac rhythm disturbances and conductivity in the clinic a number of new electrophysiological methods have begun to be employed, in particular, recording the potentials of the cardiac conducting system, stimulation of different cardiac sections, and EKG monitor recording for many hours during the activity of the subject. There has begun to be a more widespread experimental use of the micro-electrode technique to study the intracellular potentials, of tissue cultures, as well as of the method of potential fixation. As a result new data have been obtained which significantly have expanded the knowledge of the electrophysiological mechanisms for the development of arrhythmia, the effect of anti-arrhythmic preparations on the cellular electrophysiological properties, and so forth.

Currently the most widespread are the ideas that arrhythmia develops as a consequence of a disorder in the conducting of an impulse with the subsequent development of re-entry of the stimulation wave, or as a result of a disturbance in impulse formation (intensification of automatism in the ectopic center).

The emergence of an impulse in the ectopic center is related to certain changes in the transmembranous potential of action. In the first place it is assumed that the ectopic impulse can develop as a result of an acceleration in rapid diastolic depolarization. In addition its appearance is possibly governed by a reduction in the threshold potential or by an increase in the negativeness of the maximum diastolic potential at which less time is required to reach the threshold potential [11].

For the emergence of cyclic wave of stimulation of the re-entry type it is also necessary to have a number of electrophysiological conditions of which the main ones are a unidirectional blocking in one or several of the conducting paths divided anatomically or functionally, slow retrograde conducting

through the damaged path, and re-stimulation of the section located more proximally to the blockade [31, 38]. The spread of the stimulation wave over such a circle can be observed in the auricle, atrioventricular node, and ventricular myocardium, and results in the emergence of corresponding rhythmic disturbances [17, 30, 38].

The electrophysiological properties of the myocardial cell are governed by differences in the concentrations of ions outside and inside the cell. The transfer of ions through the membrane is an active process, and is executed through special channels whose opening and closing depend on the amount of the transmembranous potential. Eight channels have currently been identified. The most important are considered the potassium, sodium, and calcium channels [24, 37, 42]. On the basis of differences in the functioning of the indicated channels the myocardial fibers are divided into "fast" and "slow." The "fast" include the contractile myocardium, specialized auricular fibers, and Purkinje's fibers. The fibers of the sinus and atrioventricular nodes which are characterized by a lower transmembranous potential, a lower conducting rate, and a longer restoration time are called "slow." Damages of a varying nature can elicit depolarization of the "fast" fibers by blocking the transport of sodium, as a consequence of which they acquire properties of the "slow" cells that are dependent on the slow calcium flow, and consequently, become capable of generating ectopic impulses [12, 46-48].

There also exists the hypothesis that unites the two indicated theories for the origin of arrhythmia and proposes that on the border between the healthy and damaged tissue a current develops due to the difference in potentials. The current can result in an intensification of automatism in the border sections of the tissue, and in the appearance of conditions under which the conducting of stimulation is disrupted, and it is spread over the circle [9].

Rhythmic disturbances are observed during injuries to the myocardium of an inflammatory, ischemic, and toxic nature, disorders in the electrolyte balance, and others. The importance of these "local" factors has been proved both in experiment and in the clinic. However under conditions of a whole organism other factors as well can apparently have great importance as starters. In particular in recent years fairly extensive experimental material has been accumulated which proves the presence of the influence of the central nervous system, and some of its sections on the development of arrhythmia [7, 8, 25, 28]. It has been established that irritation of the ventromedial nuclei very often results in the appearance of rhythmic disturbances: ciliary arrhythmia, ventricular extrasystole, ventricular tachycardia, and even ventricular fibrillation which cease during the addition of irritation of the lateral hypothalamic field [8]. It is hypothesized that such action is mediated through the endocrine system since simultaneously an increase is defined in the level of catecholamines in the blood and myocardium. In tests in which arrhythmia was not successfully produced the level of catecholamines was normal [8]. However such an effect of the central nervous system on the cardiac rhythm cannot be viewed as simplified. Manning [28] showed in tests on cats that arrhythmia often develops at the moment that stimulation of the truncus cerebri ceases, but sometimes is also

observed during stimulation. Cooling the vagus promotes the interruption of such cardiac rhythmic disturbances. The importance of disorders of the higher sections of the central and autonomic nervous system including its peripheral sections in the development of supraventricular and ventricular arrhythmias is also being proved [7, 35]. All of these facts apparently indicate that arrhythmias of neurogenic origin are a result of the complex interaction of sympathetic and parasympathetic effects on the heart with a reduction or removal of the inhibitory effect of the hypothalamic system [28].

In recent years due to the use of new methods clinical electrophysiology has also begun to develop rapidly. The introduction into practice of methods for recording the electrical activity of the cardiac conducting system and the electrical stimulation of its sections made it possible to begin studying the mechanisms for the emergence of different forms of arrhythmias, as well as to begin a more detailed investigation of the state of the conducting system directly in man [2, 3, 6, 39].

In the study of patients with paroxysmal tachycardia a number of authors have shown that the extrastimulus applied at a definite phase of the cardiac cycle produces arrhythmic paroxysm in a considerable portion of such patients [40, 43, 49]. The electrophysiological basis for the supraventricular tachycardias, in the opinion of Mendez and Moe [29] is the longitudinal dissociation of the atrioventricular node into two paths (fast and slow). A tachycardial attack develops when the auricular extrasystole is conducted anterograde along the slow path which is characterized by shorter refractoriness, and then by activating the fast path which until this time has been in the refractory state it again reaches the auricle retrograde. Thus a close circle of movement of the stimulation wave is created. Currently however there is no sufficiently convincing proof that such a division of the atrioventricular node reflects functional dissociation and is not related to its anatomical division. The hypothesis exists that the fast path occurs outside the node [13]. It is possible that this viewpoint is indirectly confirmed by the observations in which it has been shown that the ventricular extrasystole sometimes provokes the emergence of supraventricular tachycardia [43-45]. Some authors believe that in supraventricular tachycardia re-entry can develop directly in the atrioventricular node without inclusion of the auricles [23], others cite data that indicates the possible re-entry of the stimulation wave in the sinus node, and sometimes even in both nodes [32]. Confirmation of these data which has been obtained in a large number of observations can prove very important in selecting the method of treatment for patients with paroxysmal tachycardias.

It is currently generally acknowledged that in patients with premature ventricular stimulation syndrome there are not less than two paths of conducting, while the emergence of tachycardia is related to differences in the rate of conducting and refractoriness in them. These differences can be constant or appear for a limited time under the influence of specific factors. In certain cases through additional paths only retrograde conducting of impulses to the auricles occurs which produce a paroxysm of tachycardia.



Here the  $\Delta$ -wave and shortening of the PQ interval are not observed. Tachycardia in the Wolff-Parkinson-White syndrome (WPW) begins with auricular or ventricular extrasystole. Depending on the path by which antegrade conducting occurs the ventricular complex will be normal or expanded [14, 18]. In the section of patients with WPW syndrome arrhythmic paroxysms are brief and easily treated, in others they acquire a serious course and are poorly susceptible to medicinal therapy. Cases have been described of sudden death during WPW syndrome combined with auricular fibrillation [14]. The known antiarrhythmic preparations, glycosides, act differently on the main and supplemental path of impulse conducting. Therefore for the selection of the correct treatment for patients with WPW syndrome a careful electrophysiological study has been shown which must help to determine where the supplemental path is located, its value in the genesis of arrhythmia, as well as by what path the impulse is conducted in tachycardia.

The disruption of the sinus node function apparently has definite importance in the development of paroxysmal arrhythmias. An example can be the syndrome of brady- and tachycardia the diagnosis of which is not complicated in the presence of pronounced clinical symptoms. Together with this in the clinic cases are probably found of the asthenia of the sinus node which is only detected under specific conditions. However the methods for evaluating the sinus node function as yet have not been sufficiently formulated [19]. For this purpose determination of the restoration time for the sinus node function after auricular stimulation by individual impulses or its rapid stimulation in the course of a short time is employed [27, 36]. The results obtained in a very limited number of observations are very variable [20]. The hypothesis exists that in the simultaneous investigation of the time for sinoatrial conducting one can obtain more complete characteristics for the state of automatism of the sinus node [41].

An attack was successfully elicited or arrested also in a number of patients with ventricular tachycardia with the help of electrical stimulation. This, in the opinion of certain researchers, is proof that the mechanism of re-entry lies at their base [43-45]. However, provocation or arresting of an attack of ventricular tachycardia in such a manner is not achieved in all the patients, which is apparently related to different electrophysiological mechanisms for the emergence of such a paroxysm, but it is possibly governed also by other not yet studied reasons. It remains unclear whether the conducting system of the cardiac ventricles, in particular the His bundle, participates in the emergence and maintenance of ventricular tachycardia, and if it does participate, then on what level does the closing of the cyclic wave of stimulation occur (His bundle, its limbs, or Purkinje's fibers). At present only in cases where the premature supraventricular impulse elicits ventricular tachycardia or arrests it, is the participation of the cardiac conducting system proved [43-45].

Diagnosis of the paroxysmal forms of tachycardias in the majority of cases does not present great difficulties since the pronounced nature of the hemodynamic disorders force the patients to seek medical help. At the same time

there exists a second group of arrhythmias--extrasystole which in many cases is not felt by the patients, and in addition is often detected only with the help of special methods [4, 5, 21, 22]. Their prognostic importance has not been sufficiently studied. As follows from the data we obtained, roughly in one-third of the individuals that did not suffer ischemia, and almost in 90% of the patients with chronic forms of ischemia ventricular extrasystoles are detected during the diurnal monitoring under conditions of the normal behavior of the subjects. It is evident from this that if the ventricular extrasystoles also have prognostic importance, then in all probability, only some of them, or in combination with other factors. Analysis of the first 300 observations we made of patients who had suffered myocardial infarction (in the program of cooperation between the USSR and the United States on problem No 5 "Sudden Death") confirmed that individuals without ventricular extrasystole, or with rare monotonic extrasystoles die from ischemia reliably less often than individuals who had polytopic or group ventricular extrasystoles. Therefore it is important to investigate the possibility of suppressing the ectopic activity with the help of antiarrhythmic drugs with their lengthy administration for the purpose of preventing sudden death. Unfortunately, despite the large number of works the problem of preventing primary fibrillation of the ventricles cannot be considered solved also for patients with acute myocardial infarction, the more so since in recent years the importance of certain extrasystoles as precursors of fibrillation in patients of this group has become doubtful [15, 26].

Determination of the reliability of these data is possible only with the help of a constant EKG recording on magnetic tape with its subsequent thorough analysis which is very important for answering the questions of the most rational tactics in prescribing antiarrhythmic preparations. At the same time the process of the physician's interpreting many hours of recording of an electrocardiographic curve is extremely labor intensive. The further successful development of research to investigate rhythmic disruptions in terms of prognosis, and its introduction into wide practice to a considerable degree will depend on the possibility of guaranteeing analysis of arrhythmias with the help of an electronic computer. Several approaches are known which are used to isolate ectopic impulses, primarily ventricular [16]. However on the whole the problem of a comprehensive EKG analysis still is not sufficiently solved and requires further treatment.

In recent years a great step has been made in understanding the nature of the effect of different drugs on the electrophysiological properties of the cell [9, 33]. The antiarrhythmic preparations were divided into five groups on the basis of these data. Their mechanism of action is presented in the works of Arnsdorf and Pinto et al. [9, 33]. Preparations of the first group (quinidine, novocainamid) reduce the rate at which sodium passes through the cellular membrane, and the rate for the conducting of stimulation. The second group includes lidocain and diphenylhydantoin (diphenin) under whose influence the transfer of potassium is increased, especially in injured fibers, and conductivity is intensified. The  $\beta$ -blockers comprise the third group. Their antiarrhythmic action is related to  $\beta$ -adrenergic blocking. In addition, it

has been shown that these preparations reduce the sodium transfer through the cellular membrane of the myocardium of the auricles, ventricles and Purkinje's fibers. The mechanism of action of the preparations in the fourth group (brethilium or ornid) has not been sufficiently studied. It is known that they do not affect the auricular fibers, but lengthen the potential of action in the His bundle and Purkinje's fibers. Isoptin is a representative of the fifth group; it blocks the transfer of calcium ions through the slow channels. The cited representatives do not have a conclusive nature [9]. There remains much unknown about the nature of the effect of certain preparations on the sinus and atrioventricular nodes, the conducting system, and the contractile myocardium, especially under pathological conditions.

The presented classification is important both for experimental work, in particular for the investigation of new antiarrhythmic substances, and for clinical practice. Knowledge of the peculiarities of their action makes it possible to make a purposeful selection of a preparation or combination of preparations of different groups.

At the present time psychotropic drugs are sometimes employed for treatment of arrhythmia [1]. The antiarrhythmic effect elicited by these substances is related to their effect on the central nervous system. However there are indications that certain of these preparations can have a definite electrophysiological effect on the conducting system of the heart. The effectiveness of treatment with these preparations, instructions for their prescription, and the mechanism of their action have not been sufficiently studied.

Despite the comparatively large number of active antiarrhythmic substances there is a group of patients in whom the rhythmic disturbances react poorly to treatment by the known preparations separately or in their combination. Often in the clinical practice patients are found in whom the attacks of arrhythmia are easily arrested by one of the preparations at the start of the disease, and then become refractory to such treatment.

It goes without saying that an investigation of the reasons for such a transformation has great practical importance. Here it is possible that new facts will be found that can expand the extant ideas in the area of cellular electrophysiology.

For treatment of forms of arrhythmia refractory to medicinal treatment, as well as in cases of the intolerability of medicines methods of electrical stimulation of the heart are currently used. The latter can be executed for the purpose of arresting an attack, its prophylactics, or only to improve the functioning of the ventricle by means of reducing the frequency of its contractions without arresting the ectopic rhythm [2, 10, 11].

Stimulation can be implemented with a frequency lower than the tachycardial frequency (from 50 to 150 impulses per minute); with a frequency exceeding the tachycardial frequency by 10-20 beats per minute, or several times, with

the help of linked impulses (artificial stimulus is triggered from the extant rhythm operator to the assigned phase of the cardiac cycle), as well single or stimuli applied periodically.

Imposition of the artificial rhythm can be temporary or constant, and occurs with localization of the problem around the endocardium, in the epicardium of the auricles and ventricles, as well as in the ventricular myocardium or coronary sinus. The developed operators of rhythm are fixed with assigned frequency or in a pattern "by requirement." The effectiveness of stimulation conducted in the works of certain authors differs significantly, which is possibly related to different mechanisms for the emergence of tachycardia, and also very probably with a number of other, yet unknown factors. It is hypothesized that a positive effect is observed in cases of tachycardia that develops according to the re-entry type [10]. Stimulation with the help of the indicated methods is ineffective in fibrillation of the auricles or ventricles of the heart. A comparatively high frequency for the restoration of sinus rhythm is recorded during auricular flutter. The lack of an effect, according to the data of certain authors, is noted precisely in those cases where one auricle generates comparatively rare and rhythmic impulses, while the other fibrillates, but as a result on the normal EKG such a combination of rhythms is evaluated as auricular flutter [10]. However this hypothesis is based on a very limited number of observations, and correspondingly requires confirmation in the works of other researchers.

An attack of paroxysmal supraventricular tachycardia is arrested at frequency from 90 to 300 and more impulses per minute. There are reports that the success of such therapy can depend on the site of catheter insertion into the auricle [11]. As yet the prerequisites are unknown which would make it possible to predict from which site the stimulation will be effective, and this question in practice is being solved by searching for this site in the process of cardiac catheterization. In order to delay the ventricular rhythm in sinus tachycardia linked, paired or very rapid stimulation of the auricles is employed. Instructions for their selection, and the effectiveness of each of the indicated methods require further study.

Currently, despite the small number of observations, there is a fairly encouraging possibility for the use of certain types of stimulation, sometimes in combination with medicines in patients with asthenia of the sinus node.

Paroxysmal tachycardias during premature ventricular stimulation syndrome, as certain cases of supraventricular tachycardia syndrome without WPW can be arrested by a single impulse applied to a certain phase of the cardiac cycle from the auricle or ventricle. Frequent stimulation of the auricles (200-220 per minute) also guarantees cessation of the paroxysm. At the same time individual observations have been described where fibrillation of the ventricles has developed in patients with an additional path of conducting impulses on the background of an attack of auricular fibrillation or very frequent auricular stimulation [11].

Temporary stimulation is evidently the most justified method of treating patients with frequently repeating attacks of ventricular tachycardia that develop on the background of transient reasons (acute myocardial infarct, acute myocarditis, intoxication, etc.). For this purpose until recently a probe has been used which was introduced into the right ventricle. Recently it has been shown that stimulation from the auricle can also arrest an attack of ventricular tachycardia, even in cases where stimulation of the ventricles is ineffective [10]. The frequency of impulses necessary to interrupt tachycardia varies a great deal and is determined in each case by experiment.

Thus, many questions concerning the use of electrical stimulation for treating arrhythmias remain unclear. More efficient approaches to therapy for rhythmic disturbances will become possible with a deeper knowledge of the mechanisms for their development.

Electrical impulse therapy of certain forms of tachycardia with the help of high-voltage defibrillators has become generally acknowledged. However data are gradually being accumulated that prove the presence of a damaging effect of such a current on the myocardium, especially in cases of multiple use [34]. The latter dictates the need to search for other methods that are effective and safe which could be used instead of an electric charge of high energy.

The cited materials indicate that electrophysiology, including its clinical part, is one of the most intensively developing sections of modern cardiology, and this, as always, is associated with a review of the extant ideas, and and the appearance of new terms, which at a certain stages begins to impair their understanding. Therefore even now the need has developed to refine many concepts used in clinical electrophysiology, and the prerequisites have emerged for a review of the extant classifications of cardiac rhythmic disturbances.

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PRINCIPAL RESULTS OF RESEARCH IN THE FIELD OF INDUSTRIAL MICROBIOLOGY

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[Article by Ye. I. Kvasnikov, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 20 Dec 77]

[Text] From the earliest years of establishment of the Institute of Microbiology, Ukrainian Academy of Sciences [UkAS], research on industrial microbiology began to develop there. It had to solve pressing problems put to science by the food and fermentation industry. At first, the studies dealt with a relatively narrow group of microorganisms, mainly lactobacilli and yeast.

There was particular development of industrial microbiology after the Great Patriotic War. It began to concentrate on several pressing problems of biosynthesis of many substances important to the national economy. The range of microorganisms included in the research broadened consistently. There was also an increase in the influence exerted by science on development of different branches of the republic's national economy.

In this article, we shall summarize the principal results of research in the field of industrial microbiology, which was pursued in this institute.

Microbiology of the Food and Fermentation Industry

Investigations of the group of lactobacilli, which are microorganisms that play an important role in many sectors of the national economy, occupy a large place in our work.

Studies of slime formation in liquid sugar media induced by *Leuconostoc* (*Leuconostoc mesenteroides*) were conducted under the supervision of M. L. Nepomnyashchaya and G. M. Frenkel' in 1933-1959. It was established that the R forms of these bacteria induce typical sliming of syrup in the production of sugar and thus cause detriment to the sugar industry. Measures to control sliming were recommended. B. O. Kagan discovered the chemistry of the process of saccharose splitting that was involved here.

Several studies were conducted on microbiology of milk; active strains of *Streptococcus lactis* were found for industrial ferments in the production

of cheeses and sour-cream [?] butter (M. L. Nepomnyashchaya and M. B. Tevelevich). It was established that starter consisting of cultures of lactic streptococcus lowers acid production in the spring and fall, due to group B vitamin deficiency of milk. The authors selected cultures that were minimally sensitive to seasonal fluctuations in milk composition, and they were recommended as components of industrial starters for sour-cream butter used in Ukrainian plants.

Staff members (M. L. Nepomnyashchaya, L. Yu. Medvinskaya, L. A. Liberman, M. B. Tevelevich, S. I. Novikova) made a comprehensive study of the effect of bacteriophagia of lactobacilli on the production of butter and cheese. It was found that the phages of lactic streptococcus are widespread in high-grade raw milk and, most often in the spring, induce lysis of all cultures contained in the starter. Sites of infection have been found at enterprises. The authors discovered seven types of *Str. lactis* phage and demonstrated that phages of types I and II are the most hazardous. It was proposed to select, from industrial and freshly isolated cultures, those that are unrelated with respect to phase sensitivity and to add to starters cultures, the population of which contains at least 20-25% phage-resistant cells. The studies led to development of measures to control bacteriophage in the dairy industry. The monograph of M. L. Nepomnyashchaya, L. Yu. Medvinskaya and L. A. Liberman, "Bacteriophage in the Dairy Industry and Measures for the Control Thereof," was published in 1961 (Kiev, UkAS Publishing House).

An extensive cycle of investigations dealing with biology of lactobacilli and elaboration of principles of regulation of their vital functions in several sectors of the national economy was conducted under the supervision of Ye. I. Kvasnikov (along with O. A. Nesterenko, V. I. Sudenko, N. K. Kovalenko, G. A. Lavrent'yeva, V. S. Podgorskiy, L. A. Mel'nichenko and Z. A. Vasil'yeva).

It was established that high alcohol resistance is one of the typical properties of the group of lactobacilli. New methods were developed for the study of lactobacilli and microbiological testing. At the present time, these methods are used widely in scientific research and industrial laboratories.

The series of studies conducted by the authors enabled them to offer a new interpretation of the ecology of the lactobacillus group and to demonstrate that lactobacilli are among the most widespread groups of microorganisms. Most often, lactobacilli develop in natural and specific industrial substrates in close correlation with organisms that have greater auxoautotrophism than they do, i.e., the capacity to independently synthesize the active substances needed for development of these bacteria.

Many industrially valuable cultures of lactobacilli can be isolated from their natural habitats.

The authors devoted considerable attention to microbiological processes occurring in biological preservation of plant materials. An active strain of lactobacillus was bred; the equipment and technological processes of reproduction thereof were developed for biological preservation of the waste from the sugar industry, i.e., pulp (in collaboration with the Institute of Botany, UkAS). The bacteria are reproduced in fermenters and, at the stage of high physiological activity, they are continuously added to pulp being stored in a warehouse, where they depress harmful microflora. This is associated with a sharp reduction of loss of nutrients from pulp and it keeps well. Adoption of recommendations tested for several years at many sugar plants yielded a significant economic effect. Good results were also obtained in industry with the use of the developed strain of lactobacillus along with cold-resistant yeast for biological preservation of apples and cabbage.

Principles were also elaborated for breeding strains of lactobacilli and using them in the meat industry for processing uncooked, smoked sausages. Lactobacilli depress development of harmful microflora in them and improve product quality.

It was found that lactobacilli and yeast adapted well to joint development in the same environment; the nature of correlations between them has been identified, and recommendations have been elaborated for controlling their development under industrial conditions.

There has been comprehensive investigation of lactobacilli, which are the pathogens of infection in a number of fermentation industries.

Recommendations were proposed, in collaboration with the Kiev Technological Institute of the Food Industry (T. P. Slyusarenko), for controlling infection in the alcohol industry. A new antibiotic was discovered (lactocid), which is produced by actinomycetes and selectively affects lactobacilli. This antibiotic depresses development of infection at alcohol plants and has a stimulating effect on processes of alcohol fermentation. Introduction thereof in industry, implemented in collaboration with the All-Union Scientific Research Institute of Fermentation Products (V. L. Yarovenko), is yielding a significant economic effect.

Lactobacilli developing in wines can be quite detrimental to viniculture. Recommendations have been elaborated for the prevention and control of diseases of wines, as well as new inspection methods that are used in the wine industry of the Soviet Union (Ye. I. Kvasnikov, G. F. Kondo, 1964).

Studies have been completed of lactobacilli of wine, which induce malic and lactic fermentation and which resulted in a new interpretation of their biological distinctions (in collaboration with L. S. Yustratova). It was shown that the direction of their metabolism is determined by their environment. The process of breakdown of malic acid is a defense reaction of

these microorganisms. It is possible to use their activity to reduce the high active acidity of wines.

It should be specially stressed that, at the present time, when sectors of the fermentation industry are changing over to continuous processes, lactobacilli, which are constantly adapting to industrial conditions, present an increasing threat to technological processes. They can lower the yield and quality of a product. Thus, we have proved that these microorganisms are often found in considerable quantities at many stages of the continuous process of champagne production. For this reason, continued investigation of lactobacilli, the pathogens of infection in the fermentation industry, and development of effective methods of controlling them are pressing matters.

The distinctions of distribution of lactobacilli in the epiphytic and rhizospheric microflora of plants have been demonstrated. For the first time, it was shown that soil is the habitat of these microorganisms, where they concentrate around the root systems of wild and, particularly, cultivated plants.

Lactobacilli, which develop in the intestinal tract of a number of warm- and cold-blooded animals, have been studied comprehensively. Atypical, motile forms of sporulating and pigmented lactobacilli, which had not been described before, have been isolated and studied.

We succeeded in demonstrating that many species of lactobacilli develop in the human intestinal tract, and they are antagonists of pathogens of gastrointestinal diseases. For the first time, it was found that the antibiotic properties of lactobacilli can be enhanced by mutagenic factors; a new antibiotic, lactobrevin, produced by *Lactobacillus brevis*, has been described.

The composition of normal microflora of the intestinal tract is often impaired (dysbacteriosis) under the influence of drug therapy, particularly with the use of antibiotics. New routes have been outlined for using lactobacilli in the control of development of harmful microflora in the intestinal tract of man and animals. We believe that it would be promising to use specially bred strains of lactobacilli with enhanced antagonism and "controllable associations" of microorganisms, the components of which would have a beneficial effect on the organism through their functions. It is recommended that such microorganisms be added in some foodstuffs.

It is interesting to mention that no lactobacilli are demonstrable in the gastric contents of essentially healthy people, and if they are found, it is in very negligible quantity. These bacteria accumulate, up to millions of cells per milliliter contents, in the presence of diverse pathological processes in the region of the stomach and duodenum, and there is marked prevalence of coccal forms over rod-shaped.

The digestive tract of cold-blooded animals is the natural habitat of lactobacilli and bifidobacteria. The number thereof depends on the species of

cold-blooded animal, stage of its development, age, diet, as well as season. Thus, it was shown that lactobacilli are a mandatory component of the intestinal microflora of useful insects (bees, silkworm), their integument and habitat. This group of microorganisms is demonstrable in amounts that warrant consideration thereof as the representative of the automicroflora of arthropods. Insects are an extremely important ecological factor in the spread of lactobacilli in nature.

Studies conducted with the use of selective media revealed many strains of lactobacilli in the digestive tract of pond and reservoir fish of industrial importance. They are referable to two genera, *Streptococcus* Rosenbach, representatives of which are isolated from virtually all tested fish species, and *Lactobacillus* Beijerinck. Tens and hundreds of millions of cells of these bacteria are contained per gram of tested material from the examined fish. In reservoir fish there is prevalence of *Lactobacillus* and in pond fish, *enterococcus*.

Many *Lactobacillaceae* and bifidobacteria are found in the intestinal tract of broilers. They are represented primarily by lactobacilli, the number of which increases in the course of formation of microflora as the chicks grow. There is a decrease in bifidobacteria as they grow. The quantitative composition of bifidobacteria and lactobacilli depends on the feed, season and raising conditions.

In the course of the study, strains of lactobacilli and bifidobacteria were found that have high antagonistic activity against several pathogens of diseases of the domestic animals examined, and they develop well in their intestinal tract. A search is in progress for effective methods of industrial reproduction of these microorganisms in order to add them to feed for prevention of diseases. These matters are acquiring particular importance in large-scale industrial breeding of both cold- and warm-blood animals, when there is a markedly increased possibility of infection.

A high-speed method of microbiological testing was developed and introduced (in collaboration with D. M. Isakova) in industrial production of acidophilus milk.

We have devoted considerable attention to problems of taxonomy of *Lactobacillaceae*. It was found possible to use the serological method for identification of lactobacilli. Methods of numerical taxonomy have been used to study coccal lactobacilli.

A cycle of studies of lactobacilli was summarized in the monograph of Ye. I. Kvasnikov and O. A. Nesterenko, "Lactobacillaceae and Means of Using Them" (Moscow, Nauka, 1975).

There was also development of work in the field of microbiology of bread baking (1935-1938). Species of yeast that infect baker's yeast, the routes of penetration thereof and influence of product quality have been described

(N. V. Stadnichenko). Studies have been made of the phenomenon of bread sliming (V. S. Rozhdestvenskiy, L. Yu. Medvinskaya). It was shown that the pathogen of slime is an aerobic sporulating bacterium, *Bacillus mesentericus*. Recommendations have been offered on bacteriological appraisal of wheat flour. Several variants of this bacterium have been described.

The microbiologists at the institute (G. M. Frenkel', M. K. Karpenko, V. V. Lipshits) investigated anaerobic bacteria in 1940-1955. They studied their physiology and variability. The authors maintain that oxygen of air is absorbed by nonmultiplying cells of obligate anaerobes. The absorption process is considered to be energetic, and the researchers believe that a certain amount of oxygen is necessary for growth and development of obligate anaerobes.

The correlations between respiration and fermentation processes may be conjugate or antagonistic, depending on the conditions under which they occur in microorganisms. The distinctive features have been established with regard to accumulation of toxins in some anaerobic bacteria. These studies were summarized in the monograph of G. M. Frenkel', "Biology of Anaerobes and Anaerobiosis" (Kiev, published by UkAs, 1956).

A study of the pathogens of acetone-butyl fermentation in *Clostridium acetobutylicum* revealed that iron ( $\text{Fe}^{2+}$ ) not only activates acetone-butyl fermentation, but alters the proportion of solvents; this is associated with an increase in butyl alcohol and decrease in ethyl alcohol (I. D. Kolchinskaya). Strictly anaerobic bacteria, which form butyl alcohol and are characterized by high amylolytic activity, have been isolated from natural substrates and submitted to comprehensive investigation (V. V. Lipshits). They were described as a new variant of butyric bacteria, *Clostridium butyricum* var. *butylicum*. Among them, an active strain was bred, which produces butyl alcohol on nonfood raw material (molasses, hydrolysates of corn cobs, sunflower hull, etc.); this strain has been tested under industrial conditions.

Research on microbiology of the canning industry resulted in establishment of the species composition of yeast microflora that causes bulging of cans with fruit; recommendations have been elaborated to refine the method for sterilizing them (N. V. Stadnichenko). The role of sporulated gas-producing bacteria in spoilage of canned meat has been studied (L. Yu. Medvinskaya).

In collaboration with the Scientific Research Institute of the Canning Industry (Ye. I. Kvasnikov, L. N. Gerasimenko), rapid methods of detecting microbial contamination of canned goods were developed and introduced in 30 canning plants.

Studies have been made of microorganisms that infect sugar products (Ye. I. Kvasnikov, A. N. Kotlyar, Z. A. Vasil'yeva). It was shown that they are

present in maximum amounts in diffusion juice. Among them, there is prevalence of the thermophils, *Bac. Stearothermophilus* and *Bac. coagulans*. A system of preventive measures has been recommended for the control of harmful microflora in sugar plants.

A cycle of studies was begun in 1963 of microbiological processes in the champagne industry and refinement of continuous processes of technology thereof (Ye. I. Kvasnikov, N. G. Sarishvili, N. G. Mel'nikov, V. F. Semenov, 1963). A new method was developed for continuous yeast reproduction for champagnizing in a unit that consists of several successively connected fermenters equipped with a system of automatic devices. A method was also proposed for reproduction of yeast in a "closed cycle," in which the yeast suspension from the last fermenter reservoir is continuously delivered in measured quantities to the first yeast fermenter through a narrow pipe. This is associated with continuous breeding of yeast adapted to the specified champagnization conditions. Thus, it was possible to breed a cold-resistant strain, *Kiyevskaya*, which ferments in reservoirs at low temperatures (5-6°C).

Subsequently, the authors (Ye. I. Kvasnikov, N. G. Sarishvili et al., 1971-1977) developed a method of reproducing yeast in a single-compartment fermenter with a turbine mixer to stir the culture fluid. Yeast is cultivated in wine with a sugar content of 0.4-0.6 g/100 ml, or without sugar, and it is used primarily to feed the ethyl alcohol in the medium. The yeast accumulating in the fermenter adapts to the activator to high pressure, low temperature and anaerobiosis, and it is delivered to the fermenter tanks. The use of this system of recommendations makes it possible to increase by several times the yield of yeast in the fermenters and to reduce sharply the size of the yeast-cultivating equipment, as well as to obtain yeast with stable, high physiological activity and to champagnize wine in the presence of an extrahigh concentration of cells thereof. This method guarantees a high-grade technological process and purity thereof. New principles have also been found for breeding strains of yeast according to an aggregate of metabolic properties for continuous champagnization of wine. The composition of asporogenic and sporulating yeasts that infect champagne production by the continuous method of champagnization has been established. It was shown (in collaboration with I. F. Shchelokova and N. P. Gal'chenko) that inadequately treated fermentation mixtures are the chief source of wine infection. It was also determined that the champagne industry is not an ecological niche for killer *saccharomycetes*.

The recommendations elaborated by the authors have been adopted at many champagne plants with a high economic effectiveness; they are also being implemented in constructing new plants in the USSR and national democratic countries.

#### Biosynthesis of Protein and Other Biologically Active Substances

Research on the use of microorganism for protein synthesis reached a global scope in the last 2 decades. Investigators are trying to breed microorganisms

and develop methods of cultivating them on media of nonalimentary raw material to obtain feed and food products that would compensate for the enormous shortage of proteins in the world. The microbiologists of the institute became actively involved in this research in the 1960's.

Attention was devoted mainly to the yeast group, since it accumulates about 50% proteins (the amino acid composition of which is close to that of animal meat), synthesizes several group B vitamins and, mainly, has a number of properties that are convenient for developing a technological process of cultivation thereof. Various sources of raw material are used for biosynthesis.

Already under the first Five-Year Plans, feed yeast was cultivated in the USSR on an industrial scale, on hydrolysates of plant waste, mainly from wood pulp. Studies in this direction are expanding. Technologists of the institute (V. F. Semenov and others) succeeded in developing regulations for growing feed yeast on the basis of diverse agricultural waste products in the Ukraine.

Sulfite liquor, the waste product of the paper and pulp industry, is one of the old sources of raw material for cultivating feed yeast. It contains carbohydrates, organic acids and other nutrients that these microorganisms assimilate and, in addition, several substances (in particular, furfural) that have a toxic effect on them. The technology of the paper and pulp industry is being constantly refined; this results in a change in composition of sulfite liquor. For this reason, the question of breeding active cultures, well adapted to multiply in changing industrial media, and which also accumulate biomass with a high protein and vitamin content, is a very pressing one. In collaboration with the Perm' Branch of the All-Union Scientific Research Institute of the Paper Industry, we (Ye. I. Kvasnikov, M. B. Tevelevich, L. P. Pantyushin and others) bred and introduced, with a significant economic effect, *Candida tropicalis* K-41A yeast and other strains, which multiply actively on sulfite liquor with high nitrogen content (so-called ammonia liquor) to paper and pulp combines. Under industrial conditions, the yeast synthesizes biomass containing 56-58% raw protein. It was shown that the yield is increased significantly with the use of biostimulators (yeast autolysate, corn extract).

The described methods of producing feed yeast (along with the method of cultivating it on mash, the waste product of the alcohol industry) are of substantial aid to the livestock industry, but cannot meet its requirements in full. In the last few years, researchers have been concerned with new raw material for biosynthesis, hydrocarbons of petroleum and natural gas, the resources of which are enormous. Major studies have been pursued at the institute, under the guidance of Ye. I. Kvasnikov (along with I. F. Shchelokova, D. M. Isakova, V. T. Vaskivnyuk, V. I. Sudenko, A. N. Kotlyar, E. F. Solomko, T. A. Grinberg, V. Ya. Masumyan, Z. I. Loyko, S. Kuberskaya, V. F. Semenov, T. P. Ostapchenko, S. R. Todosiychuk, I. D. Krivitskiy and others), to develop the microbiological bases for biosynthesis of proteins from hydrocarbons. Work was deployed on ecology, taxonomy,



physiology and metabolism of microorganisms, breeding active strains and developing conditions for growing them in continuous cultivation systems, using the method of mathematical modeling of experiments and computers.

Special attention was devoted to the study of ecology of hydrocarbon-assimilating microorganisms. Studies were made of the natural substrates in contact with hydrocarbons (as opposed to the same substrates that do not come in contact with them) and, in particular, the soil of oil and gas deposits. Quantitative cultures were made on mineral media, to which trace elements were added and individual or mixed hydrocarbons as the only source of carbon nutrients. The obtained results were expressed in absolute figures, indicating the amount of microorganisms in the tested soil, as well as ratio of microorganisms in soil saturated with oil to the amount thereof in similar soil but far from oil fields (we called this ratio the coefficient of selectivity).

All of the studies yielded coinciding, statistically reliable results. They revealed that microorganisms that consume petroleum hydrocarbons are common members of various biocenoses. In all soils, there are large amounts of microorganisms capable of oxidizing liquid and solid paraffins and, much less often, utilizing volatile hydrocarbons. There are considerably more microorganisms utilizing paraffin and aromatic hydrocarbons in soil containing oil than without it.

The breeding influence of petroleum in the soil on microorganisms that utilize different hydrocarbons is not the same. The coefficient of selectivity of soil saturated with oil for microorganisms that oxidize paraffins with fewer hydrocarbon atoms is considerably higher than for microorganisms that assimilate paraffins with a longer chain. Typically, narrowly specialized forms (which oxidize gaseous hydrocarbons; thermophiles that assimilate solid paraffins; bacteria that utilize polycyclic aromatic hydrocarbons) inhabit soil containing hydrocarbons. The obtained data contribute to our understanding of the capacity for assimilation of hydrocarbons by microorganisms in the evolutionary aspect, as a property that was formed at the early stages of their evolution.

Soil is a favorable substrate for breeding microorganisms that utilize hydrocarbons. The fewer atoms of carbon in paraffin hydrocarbons assimilated by microorganisms, the more successfully one can collect them from oil and gas fields. Microorganisms that utilize  $C_{12}$ - $C_{22}$  n-alkanes are also obtained from other habitats in relatively large amounts. When collecting these microorganisms, substrates, in which formation of industrially valuable properties is the most likely, are often preferable.

The patterns of distribution of different genera and species of yeast in oil-bearing and nonoil-bearing soil in various soil and climate regions of the Ukraine have been established.

Yeasts were isolated from soil, the phyllosphere of plants, water, plant production substrates, industrial waste, silage, cattle rumen, etc., and

identified. A collection of live cultures of yeast (about 2000 strains), referable to 20 genera and 90 species, has been created in the department of physiology of industrial microorganisms. The collection serves as base material for many microbiological investigations, especially those dealing with breeding; it also supplies strains to other institutions.

As a result of the studies, it was established that hydrocarbon-assimilating yeast is widespread in nature. Large amounts thereof are contained in oil-saturated soil. We succeeded in isolating more than 20 species of asporogenic yeast (of the genera *Candida*, *Rhodotorula*, *Torulopsis*, *Sporobolomyces*, *Trichosporon*, *Cryptococcus* and others) from oil-bearing soil in west Ukrainian fields. We also often isolated yeast that assimilates n-alkanes from reservoirs polluted with oil; there are many of them in the water of the Naftusya mineral spring; they also inhabit many other substrates. New species of yeast have been described (in collaboration with S. S. Nagornaya).

It was found that the feature of paraffin assimilation is largely related to the yeast species, and that it could be used to identify some of them.

For the first time, it was established that the capacity to assimilate hydrocarbons of the benzene class ( $C_6-C_{10}$ ), waste products of the oil industry, is inherent in several species of asporogenic yeast. Interestingly enough, these yeasts are capable of synthesizing heavy alkanes from light n-alkanes, when cultivated under oxygen-deficient conditions.

It was demonstrated that olefins can be used as raw material for microbiological synthesis in collaboration with V. S. Gutrya and R. V. Katrush).

Yeast strains have been developed, which have high growth activity on mineral media with oil hydrocarbons; their physiology and metabolism were studied. In particular, thermotolerant strain K-41 of *C. tropicalis* was bred. The biosynthetic activity of this strain and its capacity to grow at high temperatures were enhanced significantly in the course of continuous cultivation pursued for several years. The properties of the new strain have been submitted to comprehensive investigation.

It was established that yeast reacts well to the concentration of biotin in the medium by changing its growth rate. The nature of biotin requirement depends on both the source of carbon nutrients and cultivation temperature. When cultivated on a mineral medium with paraffin from the Groznenskiy Oil Refining Plant, biotin requirement is 3-5 times lower than with a medium containing glucose and hexadecane. When cultivation temperature is raised from 30 to 39°C, this yeast requires more biotin. Addition of 1.2-2.0 µg/l biotin at 39°C to a medium with paraffin increases reproduction rate by 36-40%.

Yeast cultivated at high temperatures usually experiences an oxygen deficiency. The oxygen limit can be eliminated (to a certain level) by increasing the rate of its absorption by the medium. With increase in temperature, there is

a decrease in protein content of cells and in nucleic acid content (from 8.3% at 32°C to 7.0-7.5% at 39°C).

Studies have been made of the effect of various types of oil raw material on amino acid synthesis. Valine (13-20%), leucine (14-20%) and lysine (15-20%) accumulate on media with various oil fractions in maximum amounts (of all essential amino acids).

It was shown that it is possible to develop variants of *C. tropicalis* K-41 with a higher methionine and tryptophan content under the alternating influence of chemical and physical mutagenic factors, including an electric field, the mutagenic action of which was demonstrated for the first time (studies pursued in collaboration with the Institute of Applied Physics, Moldavian SSR, B. R. Lazarev, I. B. Krepis).

It was established that K-41 yeast cultivated at 30°C contains more carbohydrates than at 39-40°C. The overall amount of carbohydrates in cells raised on paraffin is always smaller than on glucose. Glucose, mannose and traces of ribose were found among yeast monosaccharides.

The valuable patterns of lipogenesis were demonstrated in K-41 yeast, depending on cultivation conditions. It was shown that, with increase in growth rate, there is a decrease in synthesis of lipids on carbohydrate media, whereas it increases significantly on hydrocarbon media (by 5-6 times in fast-growing cells). They accumulate primarily at the expense of the phospholipid fraction (8-9-fold increase) and products of oxidation of n-alkanes--free fatty acid (5.5-fold increase). It was established that it is possible to control the lipid composition of the yeast cell both with the source of carbon nutrition and growth rate.

There is incomplete oxidation of n-alkanes and residual hydrocarbon content is 2-3 times higher in cells at high temperature, even with active growth of the culture, than at 29°C. A method of removing residual hydrocarbons from the yeast biomass by means of heat treatment was developed and tested.

It is imperative to take into consideration the increasing oxygen and vitamin requirements of the strain to assure optimum cultivation conditions at high temperatures.

The K-41 strain, which was bred and upgraded in the autoselection process, grows at a rate of the order of  $\mu = 0.3 \text{ h}^{-1}$ ; the biomass yield is about 95% and raw protein content is 52% or more. Use of the heat-tolerant strain is quite effective in the summertime, when the shortage of coolant depresses the growth of mesophilic strains.

A strain of *Trichosporon capitatum* was developed which is capable of active synthesis of protein and lipids under specified growth conditions. It is imperative to cultivate the strain under sterile conditions.

There has been investigation of the capacity to synthesize carotinoids in yeast of the genus *Rhodotorula*, isolated from various natural substrates. It should be noted that the use of a new taxonomic procedure, the hybridization method, made it possible to demonstrate the sex cycle of development in 43 out of 92 examined strains of *Rhodotorula glutinis*. For this reason, we classified these strains as a new genus, *Rhodosporidium*, family *Ustilaginaceae*, class *Basidiomycetes*.

Pink yeast strains synthesize carotinoids to different extents; most consist of  $\beta$ -carotinoids (about 50%), torulin (about 10%) and torularhodin (about 30%).

It was established that there is a correlation between accumulation of biomass and synthesis of carotinoid pigments in *Rhodosporidium diobovatum* (synonym, *Rhodotorula glutinis*), strain K-1/48. This made it possible to augment significantly the activity of the original culture by means of auto-breeding in a continuous cultivation process. The specific growth rate of the bred culture is  $\mu = 0.15 \text{ h}^{-1}$ . Protein content in the biomass constitutes about 50% and carotinoids, 300  $\mu\text{g/g}$ . Main synthesis of carotinoids proceeds concurrently with growth and continues insignificantly in the stationary phase. The mutants of strain K-1/48 obtained by the use of combined mutagenic factors synthesize up to 1000  $\mu\text{g/g}$  carotinoids.

The technology of producing yeast carotene was refined on the basis of these investigations. The developed K-1/48 strain has passed industrial trials successfully. In collaboration with the Kiev Technological Institute of the Food Industry (I. M. Royter, T. P. Slyusarenko and others), recommendations have been prepared for the use of the newly bred yeast in bread baking. The Institute of Nutrition, Ukrainian Ministry of Health, has demonstrated the safety of using these pigmented yeasts, prepared in accordance with the established regulations, in foodstuffs.

It was demonstrated that the use of "controllable associations" of microorganisms is promising in industrial biological synthesis. This is our name for microbial associations, the development of whose components is regulated in a specified direction by cultivation conditions. Beneficial results have been obtained with joint cultivation of bred strains of pigmented and nonpigmented yeasts (by regulating their growth with sources of nitrogen nutrients). As a result, valuable feed products are synthesized, which are rich in proteins, group B vitamins and carotinoids.

Several studies were pursued (together with V. V. Stepanyuk) of ultrafine structure of yeast cells developing during continuous cultivation on carbohydrate- and hydrocarbon-containing media. Studies are in progress of the phenomenon of heterogeneity of yeast cell membranes. A hypothetical scheme was proposed for the genesis of membrane structures.

A large team of Soviet scientists and industrial workers, with whom Ukrainian microbiologists also worked, mainly on the microbiological aspects of the problem, is conducting research on biosynthesis of protein from oil hydrocarbons. A team of researchers (including Ye. I. Kvasnikov) was awarded the

USSR State Prize (1971) for development of the scientific bases of protein biosynthesis from oil hydrocarbons. Production of feed yeast using refined oil paraffins is being set up in the Soviet Union on the basis of these investigations.

Many years of studies pursued at a number of agricultural and medical institutions established the degree of safety of protein-vitamin components (BVK) used to fatten livestock and standards with regard to additions thereof to the feed ration. It was also shown that the meat from animals given BVK is safe for human consumption.

The problem of developing feed protein from oil hydrocarbons is new and complex. There is still much to be done: to breed even more active yeast races; to augment the amount of protein they synthesize and utilization of nutrient components; to raise the productivity of the process; to obtain a product free of residual paraffins; to run the process under conditions that rule out completely environmental pollution.

History of technology of producing feed yeast had never known of plants with the capabilities developed in the USSR in BVK plants using oil hydrocarbons. The annual output per plant is up to hundreds of thousands of tons of BVK.

Solving the problem of producing feed yeast from nonalimentary raw material should aid in the complete eradication of protein deficiency in the diet of livestock.

Genetic studies have been made of hydrocarbon-assimilating yeast (V. P. Zharova and others). It was shown that the hydrocarbon-assimilating yeast, *C. guilliermondii*, is capable of hybridization and is referable to the heterothallic species, *Pichia guilliermondii*. The mating type of this yeast has been identified and hybrids have been obtained. In collaboration with the L'vov Department of the Institute of Biochemistry, UkAS, a genetic strain of *P. guilliermondii* has been developed, which is capable of forming a significant amount of spores (30-60%) and which presents monogenic segregation of several characters. Mutants have been developed that do not assimilate hexadecane and palmitic acid. The presence of two groups of genes controlling the "hydrocarbon assimilation" character was established in the examined culture using the complementation test. The identified genes are localized in the nucleus; "hydrocarbon assimilation" is the dominant character.

Extensive studies have been pursued of the ecology, taxonomy, physiological and biochemical distinctions of different groups of hydrocarbon-assimilating bacteria and breeding strains for the synthesis of protein and biologically active substances (Ye. I. Kvasnikov, O. A. Nesterenko, D. M. Isakova, S. R. Todosiychuk, L. A. Mel'nichenko, G. S. Yeliseyeva, V. Ya. Masumyan, Z. I. Loyko, T. M. Klyushnikova, Ye. N. Pisarchuk, N. I. Pavlenko, V. V. Lipshits, M. G. Sumnevich, A. S. Zhuravel', O. F. Petrenko).

In the course of this research, several groups of microorganisms were studied, among which cultures with important properties were found. Thus, isolated cultures of corynebacteria, nocardia and yeast capable, during submerged cultivation on mineral media with paraffins containing only an infinitesimal dose of nitrogen (starter), can assimilate the latter from the atmosphere. They utilize n-alkanes as the only source of carbon nutrition.

Interestingly enough, oligonitrophil yeast is also contained in significant amounts in the water of the Naftusya mineral spring.

Typical thermophil aerobic hydrocarbon-assimilating sporulating bacteria were isolated, presenting optimum growth at 45-65°C. A new variant was described, *Bac. circulans* var. *thermophilus*. Facultative sporulated anaerobes, referred to a new variant, *Clostridium polymyxa* var. *alkanica*, were isolated from oil wells at a depth of 800-2500 m.

Considerable attention was devoted to elaboration of taxonomy of bacteria of the genera *Corynebacterium*, *Brevibacterium*, *Arthrobacter*, *Nocardia* and *Mycobacterium*, which play an important role in the national economy, in obtaining several valuable products. Optimum media have been proposed for isolating representatives of different genera of *Nocardia*-like bacteria.

On the basis of studies of many features, in particular the cycles of cell development, ultrafine structure, types of cell division and chemistry of cells (nucleotide, fatty acid, monosaccharide composition), using methods of numerical taxonomy, we have tracked the correlations between genera and species of *Coryne*- and *Nocardia*-like bacteria, and practical keys have been proposed for differentiation between different species of *Nocardia* and saprophytic *Corynebacterium*, as well as genera of *Coryne*-like and *Nocardia*-like bacteria.

Studies have been made of ultrastructure and modes of cell division in *Arthrobacter*. It was demonstrated that division by constriction is inherent in cells that are in an active physiological state and with a septum, in cells with slow metabolism. It was established that a change in Gram staining in *arthrobacteria*, from Gram-positive to Gram-negative, is attributable to loss of external membrane layers.

An active thermotolerant strain, *Micrococcus freudenreichii* K-219, has been bred, and its physiological and biochemical properties have been studied. This strain grows very actively and is very productive.

It is important to mention that, in the course of continuous cultivation, these bacteria present the same consistent changes in synthetic processes as yeast. Thus, with increase in growth rate (from 0.2 to 0.5 h<sup>-1</sup>) on mineral media with hydrocarbons, the cells synthesize much more lipids than on media with carbohydrates, from two times more at a slow growth rate to six times more with rapid growth. There is prevalence of fractions of free fatty acids (60%), followed by phospholipids (20%), in the lipids of

bacteria cultivated on carbohydrates. In those cultivated on hydrocarbons, there are more phospholipids (28-46%), with appearance of considerable amounts of esters (20-47%, depending on the age of the culture). In bacterial cells cultivated on hydrocarbons, there are 4-7 times more phospholipids, 15 times more esters with slow growth and up to 69 times more with rapid growth, as compared to cultivation on glucose. Vitamin biosynthesis is on a high level, it does not change during growth and, converted to 1 g protein, it constitutes the following levels (in  $\mu\text{g}/\text{mg}$  protein/h): 0.018 thiamine, 0.43 riboflavin, 3.46 nicotinic acid and 0.029 pyridoxine. When scaled to one bacterial cell, the figures are as follows: 15-20 molecules thiamine, 20-30 pyridoxine, 400-500 riboflavin and 2500-3500 molecules of nicotinic acid.

The strain was successfully tested under experimental production conditions and is recommended for introduction. The newly bred race has been patented in several foreign countries.

The results of research on thermotolerant yeast and bacteria are summarized in the monograph of Ye. I. Kvasnikov and D. M. Isakova, "Physiology of Thermotolerant Microorganisms" (Moscow, Nauka, 1978).

We have demonstrated that the main property of heat-tolerant microorganisms is the capacity to multiply without substantial changes in growth rate in a range of temperatures 5-10° higher than the optimum for mesophils; the thermotolerant microorganisms are characterized by an optimum "temperature plateau," rather than a single optimum temperature point, over which growth rate and biosynthetic processes are stable or undergo negligible change.

Strains of microorganisms that have tolerance for heat, in addition to the set of industrially valuable properties, are definitely promising for use in several sectors of industry, and they merit the closest attention of researchers. In practice, the use of cultures that develop over a wide range of temperatures without a decrease in growth rate makes it possible to provide a stable technological process, even when the temperature changes. The use of heat-tolerant strains results in a saving of coolant. Finally, these strains are often more competitive than mesophils.

Several studies were pursued on the nature of correlations between yeast and bacteria when cultivated together on hydrocarbon-containing media. It was established that it is possible to obtain a higher yield of product, with higher amounts of proteins, enriched with B vitamins, including B<sub>12</sub>, which yeasts do not synthesize, when producing protein and vitamin preparations from hydrocarbons of petroleum, with the use of "controllable associations" of certain strains of yeast and bacteria, as compared to the use of monocultures; the former also permits better use of components of the nutrient medium.

Bacteria have been described that are the pathogens of yeast infection when cultivated on n-alkanes. The distinctions of their influence on yeast have been discovered, and methods have been proposed for the prevention and control of infection.

In the last few years, researchers have been concerned with problems of using synthetic alcohols--methanol and ethanol--as raw material for microbiological synthesis. Research on cultivation of yeast on methanol, which we were the first to pursue (Ye. I. Kvasnikov, Yu. R. Malashenko, V. S. Podgorskiy, V. S. Gutyrta), is being developed with success by technologists at the institute.

We have deployed research to breed ethanol-assimilating microorganisms and develop cultivation conditions on media with ethyl alcohol, using mathematical methods of planning experiments (Ye. I. Kvasnikov, V. I. Sudenko, M. N. Gavrilenko, V. G. Sumnevich, G. S. Yelisseyeva, E. F. Solomko, V. V. Stepanyuk, I. P. Stogniy, N. I. Pavlenko).

Mutants of *Rhodospiridium diobovatum* yeast have been obtained, which have high rates of growth and carotene production on mineral medium with ethanol. The mutants make the fullest use of ammonia nitrate. Iron salts have a beneficial effect on growth and carotene production. Optimum conditions have been found for cultivating yeast with high levels of proteins (50%), carotene (1000  $\mu\text{g/g}$ ) and organic iron (2 mg/g). The culture and method of running it are promising in production of items used as food and for medical purposes.

It should be mentioned that there are several difficulties involved in isolating pure cultures of ethanol-assimilating bacteria from various natural and industrial substrates. Yeasts, which assimilate ethyl alcohol, develop intensively on media containing the latter, and they depress bacterial growth. We have found that bacterial growth is virtually the same on media with low concentrations of ethanol and propanol (0.5-1.0%), and strains isolated on propanol always assimilate ethanol. A method of isolating bacteria that assimilate ethanol was proposed on the basis of these studies. To obtain accumulating cultures of bacteria, a mineral medium was used with propyl alcohol, in a concentration of 0.5-1.0%, as the only source of carbon nutrition. Transfer cultures from the former are made on agarized medium with the same formula. Subsequent replating of colonies is made on medium with ethanol.

Of the various natural and industrial substrates, the developed method was used to isolate strains of ethanol-assimilating bacteria referable to various species of the genera *Pseudomonas*, *Acinetobacter*, *Corynebacterium* and *Nocardia*. Bacteria of the genera *Acinetobacter* and *Pseudomonas* assimilate well ethyl alcohol as the only source of carbon and energy. Several strains were selected, which grow actively on medium with ethanol, one of which is *Acinetobacter calcoaceticus* K-9.

The maximum specific growth rate of *A. calcoaceticus* K-9 on a medium with 1% ethanol is up to  $0.7 \text{ h}^{-1}$ . Addition of various medium components (as they are consumed) in divided doses makes it possible to obtain a high concentration of biomass through a periodic process. The conditions for cultivating the newly bred strain were optimized by the method of mathematical planning of experiments, using a computer. In the tested factored



space, maximum productivity of the process is observed with an ethanol concentration of 4.0 vol.%, temperature of 25°C,  $D = 0.32 \text{ h}^{-1}$  and a specific amount of mineral salts.

Cultivation conditions have a substantial effect on chemical composition of cells. When grown in a chemostat culture with a carbon limit at 30°C, at a specific growth rate of 0.1 to  $0.53 \text{ h}^{-1}$  on optimized medium, there is an increase in nucleic acids (from 8.3 to 13.5), real protein level drops (from 62.0 to 54.6), there being virtually no change in wet protein (73.2-72.9%, respectively).

The biomass of bacteria cultivated on media with ethanol has a complete set of amino acids and an abundance of B vitamins, including vitamin B<sub>12</sub>. It was found that the change in morphology and ultrafine structure of cells is distinctly related to their physiological activity under various conditions of cultivating the studied bacteria on ethanol. With increase in rate of dilution of the medium there is an increase in volume of cells, and the process is described by a saturation curve. The presence of mesosomal structures is typical in cells that grow with low rates of circulation.

In all studies dealing with breeding industrial microorganisms, much attention is given to evaluation of the safety of the cultures (S. P. Rudaya). Only those found safe in experiments on animals are tested under experimental production conditions.

At the present time, the microbiological industry synthesizes protein exclusively for animal feed. A high percentage of microorganism protein is lost, and only 10-15% is used to build up protein of the animal body. For this reason, it is a major nationally important problem of producing microorganism protein for direct use as food. In this respect, the use of synthetic ethanol for microbial synthesis is very tempting.

Since 1957, several studies have been made of bacteria that produce hydrolytic enzymes (L. Yu. Medvinskaya, I. D. Kolchinskaya, N. D. Tynyanova and others). In the *Bac. subtilis-mesentericus* group, strains have been developed with high proteolytic properties. Variants were obtained under the influence of mutagenic factors, which are more active than the original ones, and their cultural and morphological properties did not undergo variability. The obtained radiation mutants have high collagenolytic and elastolytic activity; their biochemical properties are the subject of comprehensive investigation. An inverse correlation was found between catalase activity and capacity to liquefy gelatin. Strains have been obtained that are promising for use in the tanning industry for leather-softening processes. Bacteria have been found that produce proteinases when cultivated on media with paraffins.

A radiation mutant of *Bac. mesentericus* was obtained with the use of fast neutrons, which has higher proteolytic activity (A. M. Pasechnik et al.). The mutant is being used with success in the production of photosensitive materials, in the technological process of regeneration of triacetate film.

Major investigations on mycology, dealing with systematics, ecology and physiology of mycelial fungi began in the 1930's at the institute, under the influence of N. M. Pidoplichko (up to 1973) and V. I. Bilay. A strain of the new species *Penicillium vitale*, described by these authors, was found and submitted to comprehensive investigation; it actively produces glucose oxidase and catalase. The investigators (with Ye. A. Nikol'skaya), in collaboration with other scientific institutions (Institute of Biochemistry, UkAS, Ukrainian Scientific Research Institute of the Alcohol Industry, Ukrainian Scientific Research Institute of the Canned Goods Industry and others), developed industrial regulations for producing these enzymes. A shop was opened at one of the alcohol plants, on the basis of the research that had been done, to produce catalase and glucose oxidases. Catalase is used effectively at meat combines to obtain a dry protein product from the blood of slaughtered animals, and it is then used to enrich sausages. Glucose oxidases are used in medicine and for some scientific research. Studies have been made of bacteria that synthesize a number of other products (extracellular polysaccharides, wax, salicylic acid).

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We have discussed only some of the results of research in the field of industrial microbiology, which was pursued in close collaboration with many scientific and industrial organizations of the Ukraine and fraternal republics. The typical feature of this work was that there was a consistent expansion of groups of microorganisms included in the area of scientific research and refinement of methods of studying them. There was an increase in volume of studies, the results of which were introduced in the national economy. Let us outline some of the routes, along which research will develop in the future.

Work is expanding in the field of ecology. Of importance are the habitats, in which one should search for producers of specific substances, and ecological conditions enhancing formation of industrially important properties of microorganisms, in the primary collection of cultures. Ecological studies are also important in developing the means of complex processing of raw material to rule out pollution of the environment by production waste.

The increasing number of species of microorganisms used in various sectors of industry make it imperative to intensify sharply studies dealing with taxonomy thereof. Methods of molecular biology will be used extensively in the comprehensive study of physiology and metabolism of developed [bred] cultures. Research on functional morphology of microorganisms will advance to a new stage. It should disclose the distinctions of change in ultrafine structure of yeast and bacteria under specific cultivation conditions and aid in development of rational bases for cultivation thereof.

Special mention should be made of the importance of studies dealing with continuous cultivation of microorganisms. In the future too, they will develop in the direction of in-depth investigation of the properties of

newly developed cultures during the growth process and optimization of cultivation conditions. There will be considerable development of work dealing with the use of "controllable associations" of microorganisms for the production of substances important to the national economy. The use of methods of mathematical planning of experiments and electronic computer technology will augment sharply the effectiveness of the studies.

The industrially valuable properties of microorganisms will improve with the use of genetic factors on newly bred microorganisms. Development of gene engineering will yield forms with basically new, specified properties.

At the present time, along with microbiological research called upon to solve problems that face the developing fermentation and food industry, problems of microbiological synthesis will acquire increasing importance. The range of substances synthesized by microorganisms on an industrial scale will grow. With their use, increasing amounts of proteins, amino acids, vitamins, enzymes, antibiotics, hormones, lipids and other substances will be produced.

The problem of microbiological synthesis of protein is gaining exceptional importance. In our country, synthesis of feed protein is being successfully resolved on the basis of large-volume production with the use of non-alimentary raw material. Next on the agenda is development of a series of studies dealing with alimentary proteins.

The successful solution of problems put to industrial microbiology should have a profound and comprehensive influence on development of many sectors of the republic's national economy. The world of microorganisms contains enormous, still unexhausted resources.

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NEW BOOK ANALYZES METHODOLOGICAL PROBLEMS OF MEDICAL PSYCHOLOGY

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[Review by R. A. Zachepitskiy of the book "Metodologicheskiye problemy meditsinskoy psikhologii" (Methodological Problems of Medical Psychology) by K. K. Platonov, Moscow, Izdatel'stvo Meditsina, 1977, 7,000 copies]

[Text] The significance of psychology to all areas of medicine has recently been enjoying increasingly greater recognition. Associated with this is the attention being earned by medical psychology, an area of knowledge and practice at the boundary between psychology and medicine. However, successful development of modern medical psychology would be unimaginable without solution of its complex problems, chiefly those of its methodological foundations. This is why publication of this book, written by the prominent Soviet psychologist and physician K. K. Platonov and devoted to the methodological problems of medical psychology, should be welcomed.

The book consists of an Introduction and six chapters.

Chapter 1 reviews the basic problems of medical psychology in relation to their historical and logical development. The author begins Chapter 2 with the statement that definition of the object of medical psychology as pathological changes in mental processes and in the state and properties of the personality "has led to an incorrect trend exhibited by some psychiatrists who identify all medical psychology only with pathopsychology and view it as a subdivision of psychiatry." This erroneous idea is aggravated by frequent confusion of the terms "pathopsychology" and "psychopathology." The author distinguishes between these two terms. He defines psychopathology as all phenomena of the ill mind in their unity with surviving manifestations of the mentally ill personality. Pathopsychology is an area of medical psychology studying the phenomena of psychopathology; it is at the same time a subdivision of psychiatry. "The principal aspect of pathopsychology viewed as a subdivision of medical psychology is revelation of the place of psychopathological phenomena within the structure of the ill personality, and its dynamic relationship with the premorbid personality."

The author also distinguishes "extreme psychology," another of today's important areas, as a special subdivision of medical psychology, explaining that "while pathopsychology studies the abnormal personality in normal conditions, extreme psychology studies the normal personality in abnormal conditions." This is where he believes their commonness lies. Another important subdivision of medical psychology is "neuropsychology, which has already achieved worldwide recognition." The author also relates psychopharmacology to medical psychology.

In the chapter's conclusion the author dwells in detail on the areas of medical psychology that come together in the problems of labor psychology, which has acquired especially great importance today, and he presents his own original classification of the problems of labor psychology, a classification considering the basic forms of interaction between the personality and work.

Chapter 3 analyzes the methodology behind the concepts of medical psychology.

First the author defines the content of the terms "concept" and "system of concepts" on the basis of definitions given by the founders of Marxism-Leninism, and in this connection he points out that "medical psychology, as is true with all sciences, has three basic subsystems of concepts." The first and main one is the system of the fundamental principles of medical psychology, the second consists of concepts designating its role and individual problems specific to it, and the third contains its working concepts elaborating upon the objects of study, the object of medical psychology, and its relationships with other sciences.

Chapter 4 is devoted specially to the first, main subsystem of concepts--the principles of medical psychology.

Chapter 5 analyzes the personality problem in medical psychology. The struggle between materialism and idealism in viewpoints on the essence of the personality is described. Both biologization and vulgar sociologization of the personality are criticized. The author grounds the principles of the unity of personality and consciousness, the unity of personality and activity, and the structured nature of the personality.

In the final chapter, Chapter 6, the author presents his views on the methodology of medical psychology.

He examines the issue, important to medical psychology, as to the validity of using psychological tests. The author distinguishes between tests and testing, relating negatively to uncritical use of a number of foreign tests, especially projective tests, which scientists are still "vainly trying to 'adapt' to Marxist theory of personality and medical psychology." But at the same time he recognizes the need for correctly selecting domestic and foreign tests for use, recalling in this case B. M. Teplov's famous phrase that "there is no such thing as wise and stupid tests; there is only their

wise and stupid application." The author comes to the correct conclusion that if the principle of the unity of personality and activity is assumed as the basis, information on the specific features of the personality being studied, acquired by comparing data produced by different methods, including observation of the personality's activity in different conditions, and the materials of natural and laboratory experiments, including materials acquired by means of tests, must be mutually verified.

Not all problems raised by K. K. Platonov were treated with identical fullness in the book; this is obviously inevitable due to the shortness of the book. The book's great value lies in the fact that the fundamental methodological problems of medical psychology are raised and examined from theoretically valid positions, and that it notes the directions of further deeper work on these problems.

The book is doubtlessly a major contribution to development of the theoretical fundamentals of modern medical psychology. Scientists--psychologists, physicians, philosophers, and sociologists--will read it with great interest. It will also be extremely useful to practicing physicians and medical psychologists.

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PLASMIDS OF STREPTOMYCETES

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[Article by B. P. Matselyukh, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 27 Dec 77]

[Text] The research pursued in the last few decades has shown that plasmids are widespread among microorganisms. In addition to representatives of the family Enterobacteriaceae, in which plasmids were described relatively long ago and have been studied the most thoroughly [1-5], the roster of microorganisms of other species containing extrachromosomal genetic elements is growing rapidly, and at the present time it includes the genera Staphylococcus [6, 7], Pseudomonas [8], Bacillus [9, 10], Agrobacterium [11, 12], Haemophilus [13] and others.

Plasmids determine the most diverse characters of microorganisms that are essential to survival of the population under adverse living conditions: fertility, resistance to antibiotics, drugs and heavy metal ions, synthesis of specific proteins (bacteriocins), antigens, hemolysins, enterotoxins, enzymes for degradation of specific substrates (naphthalene, salicylic acid, octanes, sugars), virulence and tumor formation on plants.

Plasmids, which are capable of implementing their own transfer, as well as mobilization and transfer of chromosomal genes among various species of microorganisms, play an important role in genetic exchange, providing for plasticity of the population of microorganisms against the background of continuous spontaneous selection, thus constituting an important evolutionary factor [14, 15].

Plasmids have attracted the special attention of molecular biologists and geneticists in the last few years, in view of the use thereof as carriers (vectors) of genes from various biological sources (man, animals, yeast and microorganisms) to bacteria for the purpose of cloning, amplification and investigation of the structure and function of these genes.

Actinomycetes of the genus Streptomyces are industrial producers of most antibiotics used in medicine, and they are among the highest differentiated prokaryotes. For this reason, it is very interesting to study the plasmids

of streptomycetes to define the nature of genetic control of antibiotic synthesis, development of vectors in designing highly active antibiotic producers, determination of the role of extrachromosomal hereditary factors in the morphogenesis of this group of microorganisms.

As compared to bacteria, streptomycetes became the object of genetic research at a significantly later time. To date, there are several publications in the literature concerning extrachromosomal inheritance in streptomycetes, which contain preliminary genetic and direct biochemical and electron microscopic data on the existence of plasmids in different representatives of this genus.

Genetic data on cytoplasmic inheritance of the capacity to synthesize tyrosinase were first obtained on *Streptomyces scabies* [16]. It was shown that 99% of the spores of heteroclone colonies containing auxotrophic markers of one parent or the other produced tyrosinase regardless of which of the two parents was deficient for this character. In addition, all haploid recombinants for chromosomal markers isolated by hybridizing double auxotrophic mutants were found to be tyrosinase producers. In another study, the same authors established that acriflavine induced loss of the capacity to form tyrosine at a high incidence in several strains of *S. scabies* [17]. Okanishi et al. [18] confirmed the results of Gregory and Huang, and they also obtained new information concerning the possible plasmid control of formation of aerial mycelium and antibiotics in streptomycetes. These authors showed that colonies deficient in production of melanoid pigment (*S. scabies*, *S. venezuelae*), aerial mycelium (*S. venezuelae*, *S. kasugaensis*) and the antibiotics, kasugamycin and aureotricin (*S. kasugaensis*), under the influence of acriflavine and high temperature (35-42°C) on the corresponding strains of streptomycetes, were obtained at an incidence of 5-10%.

Using acridine dyes, resistance to oxytetracycline was eliminated from *Actinomyces rimosus* LTS-118 [19], which, in the opinion of the authors, is determined by the R plasmid. Noack et al. [20-21] observed a high incidence of stable asporogenic and inactive colonies in *S. hygroscopicus*, the producer of turomycin, under the influence of acridine orange and ethidium bromide.

More conclusive genetic evidence of plasmic control of chloramphenicol production was obtained in *S. venezuelae* [22]. Analysis of haploid recombinants from multifactor hybridization revealed that the  $ccp^+$  marker (chloramphenicol synthesis), which can be eliminated by acriflavine, could not be localized on the circular genetic map in accordance with the criterion of minimum multiple crossing over, unlike the other chromosomal markers. A high incidence of cured colonies, which lost the capacity of forming aerial mycelium, was described for *S. alboniger* after treating spores with ethidium bromide [23]. However, efforts to isolate plasmid DNA from *S. alboniger* and *S. scabies* have failed thus far.

One can only assume that plasmids exist in streptomycetes, which determine synthesis of various antibiotics, melanoid pigments and aerial mycelium,



on the basis of analysis of the cited works. The high incidence of elimination of the characters mentioned under the effect of acridine dyes or high temperature is virtually the only criterion, and an indirect one at that, of the plasmid nature of inheritance of these characters. Genetic proof of the existence of plasmids can be considered more convincing: very high incidence of inheritance of the corresponding character by heteroclone segregants or haploid recombinants [16], and the impossibility of mapping the marker because there is no linkage with chromosomal genes [22]. However, even these data are not sufficient for a definitive answer to the question.

The studies of Hopwood et al., pursued on a strain of *S. coelicolor* A3(2) constitute a new stage in research on streptomycetal plasmids; this strain has been genetically studied the most, as compared to strains of other streptomyces species (Table 1). At present, there are descriptions in this strain of two plasmids (SCP1 and SCP2) and genetic element  $\text{Chl}^R$  of transposon type (Table 2). As we have already mentioned, plasmid SCP1 has been submitted only to genetic investigation. Being an effective sex factor, it controls the fertility system. Among the strains of *S. coelicolor* A3(2), at least five types of fertility are distinguished, depending on the state of the plasmid: IF or  $\text{SCP1}^+$  (autonomous plasmid); UF, or  $\text{SCP1}^-$  (the plasmid is eliminated); NF, or two-directional donors (plasmid integrated in the chromosome in the 9 o'clock area of the map);  $\text{SCP1}^1$  (autonomous plasmid with mobilized chromosomal genes); new unidirectional donors (plasmid integrated in the chromosome).

Table 1. Comparison of genetic maps of streptomycetes

Streptomycetes	Number of mapped genes	Size of genome	Method of analysis		Literature source
			hap-loids	hetero-clones	
<i>S. coelicolor</i> A3 (2)	101	260 cM	+	+	[24,25]
<i>S. rimosus</i>	24		+		[26]
<i>S. rimosus</i> R6, R7	28	130 units		+	[27]
<i>S. bikiniensis</i>	17		+		[28]
<i>S. olivaceus</i> V KX	40	260 units	+	+	[29,30]
<i>S. glaucescens</i>	23		+		[31,32]
<i>S. species</i> 3022/a	17		+		[33]
<i>S. achromogenes</i>	15		+	+	[34]

After isolation of strains varying in fertility, data were obtained on the role of plasmids in interconversions of strains UF and IF. Vivian [36] demonstrated that, with an incidence of  $2 \cdot 10^{-5}$  for appearance of haploid recombinants, very many spores of the mixed culture (53% of all spores) contained markers of the UF parent and fertility of the IF parent. Such a high incidence of conversion of fertility from the UF to the IF type, which is 10,000 times higher than the incidence of recombination for chromosomal markers, is indicative of extrachromosomal inheritance of the fertility factor. Preservation or loss of the SCP1 plasmid does not affect the

change in direction of chromosome transfer, since both strains, UF and IF, remain recipients, as before, in the crosses with NF donors. The SCP1 plasmid transfers the capacity to produce soluble antibiotic to IF strains, preventing or minimizing development of aerial mycelium of UF strains. The data of Vivian pertaining to independent inheritance of the IF determinant and chromosomal markers in IF×UF crosses were confirmed by other researchers.

Table 2. Characteristics of plasmids of *S. coelicolor* A3(2)

Plasmid	Character	State of plasmid	DNA	Source
SCP1	1) Fertility IF type (SCP1 <sup>+</sup> ) UF type (SCP1 <sup>-</sup> ) NF type SCP <sup>+</sup> donors	Independent Eliminated Integrated Independent with mobilized chromosomal genes	Not isolated	[35-38]
SCP2 (pSH1)	Unidirectional donors 2) Methylenomycin A Fertility	Integrated Independent	Circular supercoiled and open molecules, 18-20 Mdalton	[39-41]
Cml <sup>R</sup> (Ch1)	Resistance to chloramphenicol Fertility?	Independent and integrated Transposon?	Not isolated	[42-43]

Acridine orange induces elimination of the plasmid from cells in the IF culture [45]. About 5% of the tested colonies changed to the UF type. In addition the dye prevented completely the transfer of sex factor in IF×UF crosses and was ineffective in NF×UF crosses. Thus, in IF strains the fertility factor is independent and in NF strains it is integrated.

Using a modified indirect selective method [46], which was named "plate crossing" [35] and previously used to isolated UF strains, new types of donors were obtained from IF strains, using ultrafertile strains as the test recipient [37, 38]. The new donors, like the NF and IF strains, depressed formation of aerial mycelium of UF strains, which is indicative of the presence of an SCP1 plasmid in them which, according to the authors' hypothesis, incorporated an unmarked piece of chromosome, the beginning of fixed transfer. The replaced plasmid and chromosome have homologous segments, in which it is apparently easy for crossing-over to occur, according to the model of Campbell, with mobilization of the chromosome or interaction, not yet identified, on the order of mobilization of the chromosome by the F factor in F<sup>+</sup> cells of *E. coli*. New donors appeared at an incidence of 0.17%, in the form of white sectors of gray colonies, and they transferred, with a high degree of effectiveness, the pab<sup>+</sup> marker and less so, other markers situated

along the map in a clockwise sequence. One donor transmitted marker *uraA*<sup>+</sup> effectively.

In one of the studies, an effort was made to effect interspecies transfer of the SCP1 plasmid [47]. First, UF variants incapable of depressing sporulation of UF strains of *S. coelicolor* A3(2) were selected from streptomycetal strains referable to 12 species. Of the 9 examined streptomycetes, only strain 1326 of *S. lividans* could be converted to the IF type by transferring plasmid SCP1, at an incidence of 0.6-3% of all spores in the mixed culture. There was a high incidence of transfer of plasmic SCP1 from IF to UF derivatives of strain 1326.

On the basis of the assumption that the new donors incorporated in the SCP1 plasmid had an unmarked piece of chromosome, which was next to the loci mentioned, it was interesting to find donors, in which the replaced plasmid would contain a specific chromosomal locus. Strain 1873 was obtained, under the influence of UF light and by means of plate crossing, and it transferred the *cysB*<sup>+</sup> marker at a high frequency, higher by a factor of 10<sup>2</sup> than the incidence of transfer of adjacent loci. This strain spontaneously liberates about 5% segregants incapable of transferring the *cysB*<sup>+</sup> marker. Thus, strain 1873 resembles a merodiploid and, by analogy with F<sup>1</sup> strains of *E. coli*, it was named SCP1' and its plasmid, SCP1-*cysB*. The maximum length of the chromosomal fragment contained in SCP1 equals the distance between the *adeA* and *metB* markers, i.e., about 2% of the circular genome map.

Plasmid SCP1-*cysB* was transferred from strain 1873 to strain *cys*-3 1697 of *S. lividans*. After crossing these strains, 22% of the offspring were *cys*<sup>+</sup> and contained a plasmid. They were found to be heterozygous for the *cys*<sup>-</sup> character, liberating *cys*<sup>-</sup> segregants. Reverse transfer of the replaced plasmid was less effective-- $6 \cdot 10^{-6}$  -  $8 \cdot 10^{-7}$ . Perhaps, restriction and modification of plasmid DNA or strain specificity of the conjugation process is the cause of lower frequency of interspecific plasmid transfer.

The replaced plasmids are an important tool in the hands of geneticists for specific transfer of desired genes or groups thereof to other species of streptomycetes, with subsequent manifestation of their function without integration in the chromosome.

Heretofore, only genetic evidence was submitted of the presence of the SCP1 plasmid in various states in strains IF, NF and SCP1'. Recently, an effort was made to use biochemical methods to identify plasmid DNA in strains varying in fertility. Of all the strains, regardless of fertility type, a covalently closed circular DNA was isolated, which has a molecular weight of  $20 \cdot 10^6$  [39], subsequently identified as plasmid SCP2 [41, 42] or pSH1 [40]. This plasmid plays the role of a second, less effective, sex factor and is transferred to cells of SCP2<sup>-</sup> strains [41].

Thus, heretofore it was not possible to isolate the SCP1 plasmid, which is an effective sex factor and controls synthesis of the antibiotic, methylenomycin A [48, 49].

There are 3-4 copies of the SCP2 (pSH1) plasmid per chromosome, and it contains one restriction site for EcoRI and HindIII, 2-3 restriction sites for endonucleases BamHI and PstI, and many recognition sites for endonucleases HincII, SmaI and SalI [40], which makes it possible to use it as a vector in experiments dealing with genetic engineering on streptomycetes. It will be possible to build smaller plasmids from the plasmid restricts retaining the replication starting point, and they will, perhaps, be more suitable vectors for transformation of streptomycetes. A method was developed for obtaining large quantities of plasmid DNA for biochemical studies, by means of linking pSH1 DNA with the RSF 2124 plasmid (ColEI Ap) and cloning hybrid plasmid in *E. coli* cells cultivated in the presence of chloramphenicol for amplification of the plasmid. In the hybrid plasmid, pSH1 DNA can be separated from ColEI Ap of the plasmid by treating EcoRI with restrictase [40]. Recently, some preliminary data were obtained on the existence of extrachromosomal genetic elements of the transposon type, which determine resistance of *S. coelicolor* A3(2) to chloramphenicol and are capable of more frequent transfer, independently of chromosomal genes, in crosses of strains SCP1<sup>+</sup>×SCP1<sup>-</sup> [42, 43]. In this case, the mechanism of chloramphenicol resistance is not determined by chloramphenicol acetyltransferase, which inactivates the antibiotic in other strains of streptomycetes [50-52] and bacteria, determined in the latter by R plasmids. The question of plasmid control of production of chloramphenicol acetyltransferase in streptomycetes has not yet been answered, although this enzyme is determined by chromosomal genes in *S. acrimycini* [53].

The opinion is voiced in the literature that streptomycetes may be the source of genes determining extrachromosomal resistance to antibiotics in bacteria [51]. Most natural strains of different species of streptomycetes are resistant to a wide range of antibiotics [54]. Future research will show the role of plasmids in disseminating multiple drug resistance of streptomycetes and the extent to which the DNA of R determinants of microorganisms in different systematic groups is homologous.

Systematic studies have been conducted in the department of genetics of microorganisms, at the Institute of Microbiology and Virology imeni D. K. Zabolotnyy, Ukrainian Academy of Sciences, since 1967, on the genetics of streptomycetes, which resulted in development of a circular genetic map and model of genetic recombination, as well as demonstration of bidirectional replication of the chromosome in *S. olivaceus* V KX. These studies served as the scientific basis for deploying research on the role of plasmids in genetic exchanges in streptomycetes.

Recently, plasmid DNA was isolated for the first time from streptomycetes, which determines antibiotic production and resistance to it, and which plays the role of an effective sex factor in *S. olivaceus* V KX [55] that is in second place among streptomycetes, according to extent of genetic investigation (Table 1). The molecules of plasmid DNA have the unusual shape of rosettes, which change into open circular structures under the influence of pronase [56]. The molecular weight of the plasmid is about

$30-32 \cdot 10^6$ , and apparently there are few copies thereof per chromosome. The plasmid may exist in an independent and integrated state, and it can be eliminated spontaneously or under the influence of a number of factors. Antibiotic-inactive strains, in which the plasmid is eliminated by acriflavine, ethidium bromide, ultraviolet radiation, or else spontaneously, differ from the original strains in several features: increased recombination capacity (ultrafertility) and sensitivity to several antibiotics (streptomycin, oleandomycin, polymyxin M, rifamycin and actinomycin D), altered sporulation and coloration of substrate and aerial mycelium. On the basis of these data, it may be assumed that the antibiotic produced at the early stage of culture growth, which depresses growth of Gram-positive microorganisms, regulates permeability of the cell wall and is involved in morphogenesis of streptomycetes. Perhaps it is a component of the cell wall.

The plasmid is an effective sex factor, which transfers chromosomes in conjugation. Nonselective analysis of haploid recombinants, isolated from crosses between antibiotic-inactive, ultrafertile strains and active strains, revealed unidirectional (clockwise or counterclockwise) transfer of the chromosome from donor to recipient, starting at the fixed point on the genetic map, the site of integration of the transmissive plasmid [55, 57]. Since the incidence of donor alleles in the recombinant offspring gradually declines as the distance from the transfer starting point (marker of  $ant^+$  plasmid) increases, it can be concluded that the plasmid mobilizes transfer of the entire chromosome, rather than individual donor genes. Apparently, the direction of chromosome transfer is determined by the orientation of the plasmid upon integration in the chromosome.

Thus, to date three types of fertility have been identified in *S. olivaceus* V KX: 1) UF type (ultrafertile strains with eliminated plasmid); 2) IF type (strains with independent plasmid) and 3) unidirectional donors, in which the plasmid is in an integrated state. These types of fertility were given their names because of their great resemblance to analogous types of *S. coelicolor* A3(2).

Interspecific recombination has been performed between strains of *S. coelicolor* A3(2) and *S. olivaceus* V KX, which offers an opportunity to study the compatibility of streptomycetal plasmids [58].

At the present time, research on plasmids of streptomycetes is also being deployed in laboratories of GDR, CSSR, Israel and the United States, in addition to England, the USSR, FRG and Japan.

The following generalization can be made on the basis of analysis of the few works dealing with plasmids of streptomycetes: Streptomycetes are no exception to the general rule, according to which plasmids are widespread among microorganisms of different taxonomic groups, regardless of the degree of their differentiation. Convincing genetic and biochemical evidence has been obtained of the presence of plasmids in some strains of streptomycetes, which determine fertility, synthesis of and resistance to antibiotics,

formation of aerial mycelium and other characters. The SCP1 plasmid has been submitted to genetic investigation the most, although it has not yet been possible to isolate it. In contrast, the SCP2(pSH1) plasmid has been better characterized biochemically than genetically. It is mapped by means of restriction analysis; it is cloned in *E. coli* cells as part of the hybrid ColEI Ap plasmid, and it will be used as a vector for transformation of streptomycetes. For this reason, it is necessary to develop a transformation system in streptomycetes. It was previously shown that protoplasts can be obtained in streptomycetes, as well as reversion thereof in mycelial cells [59, 60]. Moreover, transfection of actinophage DNA has been performed with protoplasts [59], as well as recombination of strains [61]. Methodologically, transformation of protoplasts by means of plasmid DNA has not yet been effected, although theoretically it is quite possible. The characters of antibiotic production and resistance to autologous and other antibiotics in streptomycetes are determined by plasmids in some cases and by chromosomal genes in others. Preliminary data have been obtained on transposons of streptomycetes.

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CIRCADIAN RHYTHMS OF PLANT RESISTANCE TO BACTERIAL INFECTION

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 2, 1978 pp 224-232

[Article by R. I. Gvozdyak, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 10 Nov 77]

[Text] Plant growth, physiological and biochemical processes in plants undergo sharp changes in the course of each 24 hours and often acquire distinct diurnal dependence. We did not encounter data in the literature about the question of whether plants are equally susceptible to bacterial infections at all times of day. For this reason, let us first dwell briefly on the theoretical premises that suggested to us the existence of circadian rhythms of plant resistance to bacterial infection and the need to confirm this hypothesis experimentally.

Interaction between plants and the environment occurs through irritability, which is a biological form of reflection of the tangible effects of the environment on the plant organism. The response is expressed as a temporary or constant relationship between the plant and environment, depending on the duration of such a stimulus. Annual and circadian rhythms of physiological and biochemical processes, corresponding to the rhythm of changes in the environment, are an example of such a constant relationship. The existence of internal circadian rhythms of growth rate, transpiration, intensity of oozing sap when plants bleed, leaf movement and other processes was known as far back as the second half of the 19th century, from the works of Sachs, Darwin, Baranetskiy and others [1].

Some interesting data were obtained from studies of growth of stock [fodder] beets. The root crop hardly grows in clear weather during the daytime, and occasionally the length thereof even diminishes. This was also found in leaves [2].

The intensity and time of exposure to light [3, 4] and temperature drop affect the circadian rhythm of movement of plant leaves and change in phases thereof, i.e., factors that have an active influence on intensity of an infectious process.

Diurnal migration of stomata has been well-studied in relation to various environmental conditions. But the same stomata serve as the portals of infection for bacterial diseases of plants.

Growth rhythms are related to the nonuniform course of physiological and biochemical processes in plants [5].

In this case, the biochemical processes and substances, to which plant resistance to disease are related, are of primary importance. In this respect, special attention is devoted to phenol compounds. There are numerous data [6-8] indicative of a relationship between quantitative and qualitative composition of phenol compounds and corresponding plant enzymes, on the one hand, and plant susceptibility to disease, on the other. Depending on conditions, phenol compounds may have a depressing or stimulating effect on pathogens of diseases. This increases even more their role in pathogenesis, since they can change from factors that retard development of parasites into factors that stimulate their development. For this reason, the natural rhythms of levels of phenols and their derivatives in a plant have particularly attracted our attention. The fluctuations of these substances in plants are so wide that their maximum concentration at night (2300 hours) is 5-6 times higher than the minimum concentration in the daytime (1200 hours) [9].

Mustard oils, that are particularly active in a free state, may be one of the factors determining susceptibility to pathogens. They can retard diseases in cabbage infected with *Xanthomonas campestris*, but do not affect the pathogens of rot referable to bacteria of the genus *Erwinia* [10]. At the same time, the quantitative and qualitative mustard oil content of plant tissue is not stable over a 24-h period.

Significant diurnal fluctuations of phytoncide [bactericide-fungicide-protozoocide (in plants)] levels are observed in plants. The maximum levels of volatile phytoncides in walnut leaves at 1200 hours are 2.5-3 times higher than the minimum at 2400 hours [11]. The minimum levels of essential oils are observed in the morning in many plants; they gradually rise in the daytime and reach a maximum in the late afternoon [12]. At the same time, the protective role of phytoncides against phytopathogenic microorganisms was demonstrated in the works of Verderevskiy [13], Bel'tyukova [14] and Galach'yan [15].

The intensity of synthetic processes, characterized by a high ratio of protein to nonprotein nitrogen, presence of certain specific proteins that are needed by obligate parasites, activity of redox enzymes in tomato leaves [8] are important factors of plant resistance to microorganisms. These processes change significantly in the course of the 24-h period.

Amino acid content is also subject to sharp circadian fluctuations in plants. Their periodic synthesis is particularly marked with regard to glycine, leucine, valine, aspartic and glutamic acids [16]. A circadian rhythm

is observed not only for quantitative, but qualitative composition. The latter is very important in the light of data on the inhibiting effect of some amino acids on parasites.

Ark [8] indicates that there is a relationship between plant resistance to bacterial infection and amino acid composition. Matyshevskaya [8] demonstrated differences in amino acid composition of tomato varieties differing in resistance to bacteria. However, in some cases, such a relationship was not observed [17].

Gasparyan [18] demonstrated that there is the most distinct change in monose and saccharose content over a 24-h period in perennial grasses. At the same time, Rubin [6] established a correlation between saccharose to monosaccharide ratio and plant resistance to infectious diseases.

On the basis of the existing data, it can also be concluded that there are circadian rhythms of absorption capacity of plants. Thus, phosphorus is absorbed much more slowly (occasionally by 2-7 times) at night than in the daytime [19]. Minimal phosphorus absorption indicates not only marked decrease in utilization of the element in the nutrient medium, but often excretion thereof by the roots. On the other hand, it was proved that mineral fertilizers and trace elements are powerful factors in enhancing plant resistance to diseases [20, 21].

Such marked circadian fluctuations of physiological and biochemical processes in plants are consistent with the effects of physical endogenous factors on them. Thus, Biebl and Hofer [22] found that there is a 1.5-2-fold change in radioresistance of the epidermis of onions and moss leaves. Maximum radioresistance of onion epidermis cells was observed at about 1200 hours and minimum, at 0800 hours (at 0400 and 2000 hours, respectively, for moss); there was a correlation between radioresistance and change in nucleus size in onion cells.

The circadian rhythms of physiological and biological processes have not been sufficiently investigated in microorganisms and hardly studied in bacteria.

Fifty years have passed since the correlation between growth regions of fungi [or mushrooms] and time of day was first demonstrated; at the present time, the role of nucleic acids in this process has been proved.

Ogato et al. [23] tried to demonstrate circadian rhythms of growth rate of bacteria inoculated between midnight and 0500 hours. There was a 3-fold difference in growth rate at the maximum and minimum periods.

A culture of *Proteus vulgaris* inoculated at 0800 hours differed from a culture inoculated at 2000 hours [24]. The former grew faster and produced less ammonia. The authors believe that an X factor, yet unknown, influences this process.

On the basis of the data in the literature concerning circadian rhythms of physiological and biochemical processes, on the one hand, and relationship to them of plant resistance to infection, on the other, we assumed that there are also circadian rhythms of resistance to bacterial diseases.

An effort was made to confirm experimentally the possibility of such a correlation with regard to bacterial pathogens of plant diseases, and the results thereof are summarized in this article.

Experiments to elicit the influence of infection time on susceptibility of plants to bacterial infection were conducted on Havana variety of tobacco infected with *Pseudomonas tabaci* (strains 223 and 42), Nezhinskiy variety of cucumbers infected with *P. lachrymas* (strains 6-5 and 7591), on beans infected with *Xanthomonas phaseoli* (strain 2), Mayak and Sovetskiy tomatoes infected with *X. vesicatoria* (strain 9p) and on beech trees infected with *Erwinia nimipressuralis* 362 and *E. rhapontici* (445-3 and 418-3). The strains we used were obtained from a collection of live cultures, with the exception of 362, 445-3, 418-3 and 6-5, which we isolated; strains 2 and 9p were kindly provided by V. I. Samoylenko and M. S. Matyshevskaya, to whom we are sincerely grateful. Experiments were conducted at the experimental center of the Institute of Microbiology and Virology, Ukrainian Academy of Sciences (Kiev Oblast) and in the Carpathian region. They were repeated 4-12 times.

When studying the correlation between plants and bacteria as related to the time of initial contact, one must take into consideration the fact that there is interaction between two organisms, the activity of which may change within this time. We infected tobacco and cucumbers with 1-day and 3-day bacterial cultures to avoid mistakes. It was first determined experimentally that the virulence of the bacteria was unrelated to the original age of the culture. At the same time, phytopathogenic bacteria applied to the leaf blade at different times of day induce a pathological process that differs in intensity. For example, during flowering, the susceptibility of tobacco leaves to *P. tabaci* presents a double-peaked curve (Figure 1). The first, more marked maximum of susceptibility during the flowering period is observed at 0900-1100 hours and the second, at 1500-1700 hours. This is typical for the more virulent strain, *P. tabaci* 223.

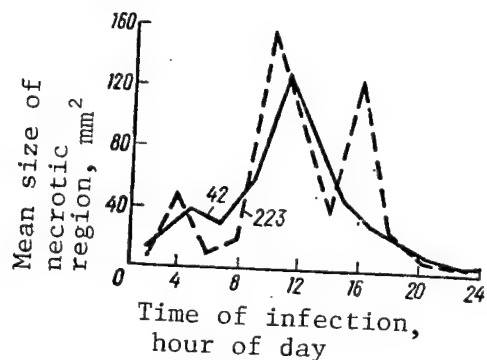


Figure 1.  
Susceptibility of Havana tobacco leaves to *Pseudomonas tabaci* (strains 223 and 42) over a 24-h period. Level of significance of difference between maximums as compared to infection at 0800 hours:  $P < 0.05$

More extensive experiments were conducted on Nezhinskiy cucumbers using highly virulent strains of *P. lachrymas* (6-5 and 7591). Observations were pursued for 3 years [25, 26] at different stages of the season. Plant resistance was determined on the basis of mean size of necrotic spots and number of infection sites manifested (out of 125 that were possible).

The common finding for leaves on all levels is that their susceptibility to bacterial infection changes in the course of the day: it is lowest at night and highest in the daytime. However, when the 4th and 5th layers of cucumber leaves were infected, although susceptibility changed sharply at night, as compared to daytime infection, it still remained rather high. Nocturnal susceptibility was negligible in older leaves, particularly the lowest level thereof; in fact, instead of large necrotic regions inherent in angular leaf spot in cucumbers, there is appearance of small necroses at the injection sites. Increased sensitivity to infection was observed at dawn (0500 hours) in leaves on all levels. Subsequent development of disease depends not only on the time of infection, but number of levels, and this can be seen particularly distinctly from the time of optimum susceptibility of leaves. Thus, development of infection is the most intensive when leaves are infected at about 1500 hours for the first and second levels and at 0900 hours for the leaves on the third to fifth levels.

There is a significant increase in susceptibility of cucumber leaves on the fifth level, as in tobacco leaves, in the predawn hours, before sunrise (0400-0500 hours). Such a sharp change in resistance at this time cannot be attributed solely to elevation of temperature, which changes insignificantly at this time, sharp increase in solar radiation or the effect of increasing intensity of earth's magnetism. We conducted our studies under natural conditions, so that these factors cannot be entirely ruled out. However, in the leaves on the lower levels of cucumber plants, we find less susceptibility to infection at this time. It may be assumed that the enzymatic systems of leaves of different ages react differently to physical factors, and this is reflected as well in their susceptibility. We consider it plausible that the temperature factor does not play a decisive role here, since some experiments were conducted at night, at a temperature of 18-20°C. This is also confirmed by the observations made in 1969, in experiments conducted under the same conditions with cucumbers 1.5 months of age, on the warm days of late June and considerably colder ones in September. The overall curve of circadian susceptibility was virtually identical, regardless of season when the plants were infected.

This pattern is unrelated to the age of the cucumbers, season or weather. Only the nature of the curve may change: occasionally it has two peaks, in other cases the peaks are shifted, but we never observed identical susceptibility of cucumbers to the pathogen of angular leaf spot, *P. lachrymans*, in the course of a 24-h period.

Circadian rhythms of plant resistance to bacterial infection were also confirmed on the example of II Pyatiletka variety of beans and the pathogen



*X. phaseoli* 2 (Figure 2) [27]. Plant leaves were infected at the flowering stage, leaves and beans were infected during the stage of fruit development.

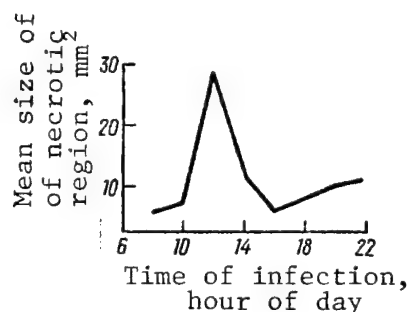


Figure 2.  
Susceptibility of II Pyatiletka bean leaves to *X. phaseoli* over a 24-h period. Level of significance of difference between infection at 1200, 1400 and 2200 hours, as compared to 0800 hours:  $P < 0.05$

Lowest resistance of bean leaves to *X. phaseoli* was observed at 1200-1400 hours, after which it increased sharply and then decreased again. This pattern was observed after infecting the plants at the flowering and bean-forming stages. Beans react analogously, the only difference being that the peaks of decreased resistance are shifted in time and more distinct in the second half of the day.

Experiments involving infection of Mayak and Sovetskiy varieties of tomato leaves and fruit with *X. vesicatoria* also demonstrated the existence of two peaks of susceptibility: one at 1000-1200 hours and the second at 1800 hours (Table 1, Figure 3).

Table 1. Susceptibility of Mayak and Sovetskiy tomato leaves to *X. vesicatoria* as related to time of day of infection

Time of infection, hour	Necrotic injury zone, %*	
	Mayak	Sovetskiy
8	100	100
10	113	187
12	142	160
14	118	84
16	54	158
18	118	159
20	93	86
22	84	

\*The necrotic region developing after infection at 0800 hours was taken as 100%.

In tomatoes, as in cucumbers, the leaves of the lower levels are less susceptible than on the higher ones. However, no difference in dynamics of susceptibility of leaves to *X. vesicatoria* is observed between the levels.

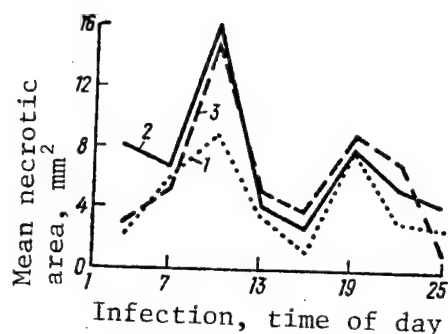


Figure 3.  
Susceptibility of Mayak tomato leaves to *X. vesicatoria* 9p over a 24-h period. The numbers refer to leaf tiers. Level of significance of difference between infection at 1000 and 1900 hours, as compared to 0700 hours:  $P < 0.05$

We checked the diurnal dynamics of resistance of beech leaves in a 5-6-year growth in a beech forest about 100 years old in the Krivulya area of the Mukachevskiy forestry of Zakarpatskaya Oblast.

The obtained data (Table 2) are indicative of different susceptibility of beech leaves in the course of a 24-h period. This pattern, common to all three strains, differs in essence only by the numerical indices, size of necrotic spots, depending on virulence. The disease is manifested the soonest when leaves are infected at 1100 hours. Already 7 h after infection we see lesions. The maximum size of the lesions after infection at 0900 and 1100 hours persists throughout the observation period and, for example, it is 3.5-5.4 times larger after infection with the highly virulent strain *E. nimipressuralis* 362 and the less virulent strain 418, as compared to the necrotic region after infection at 0700 hours. After infection at 1300 hours, there is some increase in plant resistance, but it again diminishes by 2-3 times after infection at 1700 hours, as compared to 0700 hours.

Table 2. Diurnal dynamics of beech leaf resistance to *E. nimipressuralis* 362 and *E. rhapontici* 445-3, 418-3 (infected on 31 May 1969)

Time of infection hour	<i>E. rhapontici</i>					<i>E. ntimpressuralis</i>		
	445-3		418-3			% pos. inocul. out of 70 (1 Jun)	necrosis, mm <sup>2</sup> (13 Nov)	
	% posi- tive inocu- lations of 125 (1 Jun)	nec- rosis, mm <sup>2</sup> (13 Nov)	% pos. inoc. out of 125 (1 Jun)	necrosis, mm <sup>2</sup> (13 Nov)				
				M±m	P			
7	0	0	0	5±1		10±3	6±1.5	
9	7±3	6±1	23±5	11±1	<0.05	90±5	81±17	<0.01
11	14±4	19±2	59±6	27±4	<0.01	90±3	135±21	<0.05
13	9±3	9±1	27±5	7±1	>0.05	77±5	34±7	<0.01
15		11±2	16±4	8±1	>0.05	69±5	20±5	<0.01
17		8±1		14±3	<0.05	16±4	13±2	<0.01
19		24±1		7±2	>0.05		17±4	<0.01

Note: *P* refers to level of significance of difference as compared to infection at 0700 hours.

The range of susceptibility of plants in the course of a 24-h period is not related only to the physiological and biochemical state of plants at a given time, but to extent of bacterial virulence. Against the general pattern of change in resistance, which is unrelated to virulence of bacteria, the more virulent strain 362 of *E. nimipressuralis* induces necrosis 2 h sooner than less virulent ones.

Moreover, plant resistance is so high after infection at 0700 hours, that it can be unsurmountable for the mildly virulent strain *E. rhaiponici* 445-3, whereas after infection at 1100 hours rather large necrotic areas are formed (19 mm<sup>2</sup>).

Let us mention that development of the infectious process is faster after using less virulent strains for infection at 1100 hours than at 0900 hours, whereas in the case of the very virulent strain formation of necrosis is faster after infection at 0900 hours.

However, we cannot attribute the significant persistence of retarded development of infection observed immediately after infecting the plants solely to the change in plant resistance over the 24-h period. Perhaps, in our case, there was an increase in plant resistance, similar to the one observed with the use of highly and mildly virulent strains of bacteria. In our experiment, the pathogen itself enhances plant resistance, after penetrating at the time of high resistance.

We cannot rule out the possibility of effects of antibiotics on the pathogen and hence the significant decline in original number of bacterial cells. In this case, considering the high reproduction rate of bacteria, other conditions being equal, which occurs on subsequent days in the plants in all experimental variants, we should have expected equalization of severity of lesions, which is not actually the case. For this reason, the first explanation appears more likely to us.

On the basis of the submitted data, we can derive the general conclusion that there are in nature near-diurnal rhythms of plant susceptibility to bacterial infection. The common finding for all tested plants was the sharp increase in resistance at night, as compared to the daytime. During the day, we usually observe two peaks of decline in resistance. The first (at 1100-1300 hours) is more distinct than the second (1600-1800 hours).

Our next task was to investigate the effects of antibiotics on natural rhythms. Studies were pursued on the cucumber, with the pathogen, *P. lachrymans* and the antibiotic, streptomycin. We selected this antibiotic on the basis of preliminary experiments, which revealed that, of the 11 tested strains, strain 605 of *P. lachrymans* was the most sensitive to this antibiotic.

The studies confirmed the high activity of streptomycin. In our experiments, antibiotic-treated plants were 3-12 times less sensitive to infection than

the control. However, even under these conditions, the circadian rhythm of plant susceptibility to bacterial infection persisted, though it was less marked.

Regardless of the near-circadian rhythms of susceptibility, the time of day at which plants were treated also affected the efficacy of the antibiotic. The best time to treat cucumbers with streptomycin is before 0800 and at 1800-2000 hours. Plants treated at this time became the least sensitive to the pathogen, and there was more than 10-fold decrease in their susceptibility to *P. lachrymans*.

We believe it promising to continue investigation of the effectiveness of antibiotics on plants, depending on the time of day they are given.

The obtained data enable us to offer a few recommendations. Thus, when making a comparative study of resistance of different varieties of plants to bacterial infection by the method of artificial infection, one cannot infect the plants all day long, without a special control. A plant of one of the varieties, which must be infected every 2-3 h, may serve as such a control. One should compare the resistance of the varieties to that of the control only within this time.

The same applies to testing the virulence of many bacterial strains; in this case, one of the strains can serve as the control. It is also important to choose the time for testing the pathogenicity of freshly isolated bacteria. This applies particularly to mildly virulent isolates, the virulence of which may not be demonstrated at all if the plants are infected during a time of increased resistance. For some pathogens and plants, it is best to experimentally determine the time of maximum plant susceptibility and conduct the tests at that time.

One must also take the circadian rhythms of plant resistance to bacterial infection into consideration in evaluating the harmfulness of insects, which are vectors of plant disease pathogens. It is important to take into consideration not only their number, but activity (of the insects) in the course of the day. Disease-carrying insects that strike plants at the time of maximum susceptibility to pathogens will inflict more damage than those that attack plants at a time of least susceptibility. For this reason, the fact that some insects prevail in number does not necessarily mean that they are the most harmful as carriers of diseases.

The diurnal difference in plant resistance to bacterial infection may also be used to answer some questions of correlations thereof. In order to determine the significance of specific processes, substances, etc., to plant resistance, we consider it best to use one species and variety of plant, rather than various varieties with certain other properties.

By studying metabolism in one variety, but at different times of day with regard to plant susceptibility to infection, we can rule out the objective

mistakes that are related to varietal differences, and which are not responsible for plant resistance.

Thus, for the first time, there is experimental proof of the hypothesis we expounded to the effect that plant resistance to phytopathogenic bacteria fluctuates in the course of a 24-h period.

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HIGHER PLANTS AS A SOURCE OF NEW ANTIBIOTICS

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 2, 1978 pp 233-241

[Article by B. Ye. Ayzenman, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 2 Nov 77]

[Text] Development of the antibiotic problem yielded results of the utmost value to medical practice, and it constituted a major contribution to general biology. Microscopic fungi, actinomycetes and bacteria were found to be a source of biosynthesis of antibiotics. For a long time, higher plants had been studied very little in this aspect. Yet, plants had been used in folk medicine for a very long time in the treatment of various diseases, including some that could be suspected of being infectious.

But it is only after B. P. Tokin discovered the phenomenon of phytoncides [bactericide-fungicide-protozoacide] that the teaching on antibiotic properties of higher plants acquired a scientific foundation and drew the attention of numerous researchers.

Development of antibiotics from higher plants is one of the aspects of research on phytoncides. In this article, we submit some of the results of studies conducted in the department of antibiotics, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, dealing with the antibiotic properties of plants, which were begun in 1946 at the initiative and under the supervision of V. G. Drobot'ko; B. Ye. Ayzenman has supervised them since 1963.

One of the most important prerequisites for such research was to study plants in the taxonomic aspect. Determination was made of the presence and incidence of phytoncidal properties in plants representing numerous genera and species of different families. Since synthesis of some antibiotics is inherent in plants of specific families (for example, protoanemonin is synthesized by plants in the family Ranunculaceae, isothiocyanates by plants of the family Cruciferae, etc.), demonstration of high activity in plants referable to these and related families could aid in tentative evaluation of the nature of antibiotics they produce. On the other hand, detection of plants with antibiotic activity other than is inherent to species of a



given family could serve as an indication that they biosynthesize some other, possibly new, antibiotic.

Studies were conducted with consideration of the extent of accumulation of antibiotic substances in plants, as related to their physiological state and ecology (age, stage of development, habitat, effects of seasonal factors, etc.). The distribution of antibiotic substances in different plant organs was examined. A comprehensive search was made of the literature pertaining to chemistry of the plants studied. This also aided in defining their antibiotic properties, since some nonspecific compounds contained in plants also have varying degrees of antimicrobial activity and, if present in a significant quantity, could cause errors in demonstration of antibiotic producers.

Studies were made of the antimicrobial, less often antiviral and antineoplastic properties of various products obtained from higher plants in our department. We studied the flora of the Ukrainian SSR, Turkmen SSR and part of Northern Caucasus. We also tested antibiotic activity of some substances of plant origin (alkaloids, glycosides and others). Antibiotics were isolated from the active plants that were discovered; they were submitted to chemical and biological investigation, and determination was made of their potential use to medicine and agriculture; effective products were introduced to practice.

Since the performance of all these tasks required the involvement of various specialists, we conducted our research in conjunction with other institute departments, many scientific research and practical institutions of the UkSSR and other republics of our country.

#### Antimicrobial Properties of Higher Plants

Determination was made of antimicrobial activity of 1500 plant species. Studies of aqueous, alcohol, acetone, ether and, less often, other extracts revealed that virtually all plants contain substances with antimicrobial properties, though the spectrum and level of activity varied. In the vast majority of cases, the extracts affect only Gram-positive bacterial species, they affect fungi less often and Gram-negative bacteria very seldom. Of the latter, *B. pertussis* is the most sensitive.

Studies were also made of essential oils obtained from many plant species. Only a few of them presented high antimicrobial activity.

We studied antimicrobial activity of many alkaloids: pure products obtained from the All-Union Scientific Research Chemico-Pharmaceutical Institute (Moscow), chair of plant physiology of the Uzbek University, Institute of Chemistry, Uzbek Academy of Sciences, as well as unpurified preparations, i.e., total alkaloids isolated from many plants, which were studied in the department of biochemistry of microorganisms of our institute. Antimicrobial activity was found in many alkaloids, but there were very few highly active preparations.

Some glycosides, organic acids and tannic substances also presented antimicrobial activity, though it was relatively mild.

Nevertheless, some of the above groups of substances determined the antimicrobial activity of a number of plants. For example, it was established that the high antimicrobial activity of the yellow water-lily (*Nuphar luteum*, Nymphaea family) is attributable to nupharin, originally isolated from the plant as a new antibiotic, but upon comprehensive chemical investigation (S. I. Novikova) it was found to be a long-known alkaloid, whose antibiotic properties were not known previously.

The antibacterial action of many plants was found to be attributable to gallic acid.

Highly active preparations were isolated from a number of plants, some of which have been named and others, not, and each has been studied to varying extents. We shall discuss only some of them.

Antibiotics from common St. John's wort (*Hypericum perforatum*, Guttiferae family): Two complex antibiotics, imanin and novoimanin, and one in an individual form, the new antibiotic hyperforin, were isolated from this plant.

Imanin differs from novoimanin in method of isolation, appearance, chemistry, biological action and therapeutic efficacy. Both affect Gram-positive bacteria, including antibiotic-resistant staphylococci. Novoimanin is considerably more active than imanin, it affects staphylococci when used in a concentration of 0.1  $\mu\text{g}/\text{ml}$ , versus up to 4  $\mu\text{g}/\text{ml}$  for imanin.

Imanin (isolated by P. I. Kisel' et al.) is a product consisting of many components, including antibiotically active ones. The product also contains chlorophyll, products of its hydrolysis, tannic and other substances. Two main antimicrobial substances were isolated from imanin, but they were found to be very unstable when stored and were not investigated definitively. Imanin has low toxicity and was used for about 20 years in medical practice. Its properties have been well-described in the literature. Imanin is particularly effective in the treatment of severe burns: it reduces loss of plasma, dries tissues, lowers intoxication, accelerates processes of tissue regeneration and often prevents formation of tight, ugly scars. It is very effective in plastic surgery on noninfected wounds. A. D. Bobyr', who conducted observations for many years over wide areas, established the high efficacy of imanin in the control of viral diseases of some *Solanum* plants, induced by the viruses of tobacco mosaic, big bud and bronzing; imanin lowers morbidity, increases harvest and quality of tobacco. The State Commission under the USSR Ministry of Agriculture has recommended this antibiotic for practical use in prevention of tobacco mosaic, bronzing and big bud.

Novoimanin (obtained by N. A. Derbentseva and A. A. Rabinovich) has been produced by the medical industry for about 15 years; it is used in clinical

medicine as a topical agent for severe, suppurative local processes due to staphylococcus (infected wounds, osteomyelitis, abscessing pneumonia and pyopneumothorax, some urological and ENT diseases, including chronic tonsillitis and others). It is highly effective in pediatric surgical practice, especially in the treatment of infants. Although novoimanin is produced and recommended as a product for external use, there has been expansion of indications for use thereof in clinical practice. This made it necessary to make an additional, comprehensive study of the properties of novoimanin, including pharmacodynamic and several others (P. S. Volosovets). It was shown that novoimanin penetrates into blood and organs in an antibiotically active state after some routes of administration (aerosol and others); its antimicrobial and therapeutic effect on the lungs was demonstrated. This product also has a beneficial effect on defense mechanisms of the organism; it stimulates immunogenesis. It was established that novoimanin has no allergenic or antigenic properties (P. S. Volosovets, S. R. Reznik). A substance was isolated from novoimanin, then from the St. John's wort plant (N. A. Derbentseva), which determines their antimicrobial activity studied at the Institute of Bioorganic Chemistry of the USSR Academy of Sciences. This new antibiotic, hyperforin ( $C_{35}H_{52}O_4$ ) is a polyterpene compound consisting of a bicyclic tetraketone with four isoprenoid chains (A. I. Gurevich et al., 1971). Hyperforin is more active and less toxic than novoimanin, but it is very unstable; it breaks down very rapidly (within a few days), losing its chemical individuality and antimicrobial activity, whereas novoimanin is quite stable during storage.

In collaboration with the department of biochemistry of microorganisms, we isolated the antibiotic, arenarin, from the immortelle (*Helicrysum arenarium*, Compositae family). In the opinion of Ye. Ya. Rashba, its chief antimicrobial element is 5,7,4-trihydroxyflavanone (naringenin). Arenarin affects only Gram-positive bacteria; it has anti-inflammation properties, stimulates defense mechanisms of the organism, accelerates regeneration of tissues, and it has low toxicity (A. K. Negrash). Clinical studies revealed that it is highly effective in the treatment of chemical and thermal burn injuries to the eyes. Arenarin is permitted for use in ophthalmology, and industrial production thereof is being organized.

Previously, arenarin was tested extensively in plant growing, for the control of bacterial canker of tomatoes, the pathogen of which, *Corynebacterium michiganense*, was found to be quite sensitive to this antibiotic. The many years of observations of D. I. Bel'tyukova, M. D. Kulikovskaya and others, covering large areas, revealed that the number of sick plants is reduced and the harvest increased when tomato seeds are treated with arenarin.

Arenarin was recommended by the State Commission under the USSR Ministry of Agriculture for use in plant growing.

Salvin is an antibiotic obtained by N. A. Derbentseva from the medicinal plant, garden sage (*Salvia officinalis*, Labiatae family). Other sage species are not very active (N. A. Derbentseva, S. A. Matveyenko). Salvin

affects Gram-positive bacteria; it induces very rapid (within 5 min) extinction of staphylococcus and hemolytic streptococcus in vitro and in the mouth and throat (it also reduces sharply other microflora), but after 20-30 min these bacteria begin to be demonstrated again, their number gradually increases, probably because they enter the above areas from adjacent cavities and inhaled air. Two chemically similar antimicrobial agents, which determine the activity of salvin, have been isolated from the latter in individual form. It was established that they are referable to diterpene acids (Institute of Bioorganic Chemistry, USSR Academy of Sciences): one of them is picrosalvin (carnasolic acid) and the other is a methylated derivative of picrosalvin, methylpicrosalvin.

Salvin has low toxicity and was found to be an effective agent in the treatment of many diseases in stomatological practice. At the present time, salvin is being adopted in medical practice and introduced to the medical industry.

Preparation K was isolated by A. S. Bondarenko from plants referable to the Compositae family. It depresses growth of Gram-positive bacteria and fungi, demonstrating selective activity in dermatophytes. Substrates have been isolated from preparation K, which determine its antibiotic activity: component 2f, an isolated substance, the structure of which was established at the Institute of Chemistry of Natural Compounds, USSR AS [Academy of Sciences] and a complex of oxygen-containing substances, the chemistry of which was studied at the Botanical Institute of the USSR AS.

Preparation K has low toxicity. It was established that it is highly effective in experimental microsporia of guinea pigs and visceral epidermophytosis of white mice (G. T. Petrenko, Ye. K. Khayetskiy), when used both topically and internally. When given per os, preparation K is rapidly absorbed in the blood, distributed in organs and excreted in urine (T. I. Skorobogat'ko). It was submitted to the pharmacological committee of the USSR Ministry of Health to obtain permission for clinical investigation of use in the form of ointment for dermatomycosis.

Several antibiotics were isolated from Psoralea drupaceae (Leguminosae family) plants (A. S. Bondarenko). In collaboration with the Botanical Institute, USSR AS, it was established that the active element of all psoralea products is the monoterpene phenol, bakuchiol, which was isolated at almost the same time as we did, in 1973, in India by Mehta et al., from the seeds of another species, Ps. corilifolia. A. S. Bondarenko was the first to discover the antibiotic properties of bakuchiol, as well as Psoralea drupaceae. Bakuchiol has a high antimicrobial activity and marked selectivity of action: it depresses growth of Gram-positive bacteria, including all antibiotic-resistant staphylococci, in doses of 1-5  $\mu\text{g}/\text{mL}$  and dermatophytes, in doses of 2-20  $\mu\text{g}/\text{mL}$ . A concurrent study is being pursued of the biological action of bakuchiol and all complex products containing it (for the purpose of selecting the most promising one for practical use), since the activity of the latter is also probably attributable to minor components, while the method of obtaining them is much simpler than for bakuchiol.

Of the other, less studied antibiotics, let us discuss only a few. We isolated cansatin from wild, cultivated and Indian hemp. Its activity is determined by cannabidiol substances; it affects bacteria and plant nematodes. Helorgin is a highly active crystalline product isolated from *Heliantus orgyalis*, Compositae family, in an individual form; it has not yet been studied chemically; apparently, it is a phenol; it affects Gram-positive bacteria and spores of white blister [*Ustilago maydis*] of corn. Rafin (a mixture of acid and phenol substances of the plant) was isolated by A. K. Negrash from the black radish, and it has several valuable qualities, but has been little studied as yet.

As can be seen from the submitted data, substances determining antibiotic action were isolated from virtually all complex antibiotics of plants; it was determined that they belong to specific groups of chemical compounds, and the chemical structure of some of them has been identified.

The mechanism of action was studied in only some antibiotics. For example, there have been studies of some aspects of the mechanism of action of imanin and novoimanin. These antibiotics alter the morphology and cultural properties of staphylococcus. According to the data of N. Ye. Preobrazhenskaya, imanin depresses oxidation of amino acids by staphylococci; novoimanin disrupts the Krebs cycle after the stage of conversion of  $\alpha$ -ketoglutaric acid. In the author's opinion, novoimanin contains inhibitors that dissociate bacterial respiration and phosphorylation. According to the data of T. I. Ivanova, Ye. K. Khayetskiy and G. T. Petrenko, preparation K has a deleterious effect on fruit-bearing organs of *Trichophyton gypseum* and *Microsporum lanosum*. For example, under the influence of subfungistatic doses of preparation K, *M. lanosum* is deprived of its most typical species-specific character, multichamber macroconidia (spindles). Preparation K and the components determining its activity (individual substance 2f and the fraction of oxygen-containing substances) have a different altering effect on the fraction composition of fungal proteins, their electrophoretic motility; they depress the activity of some enzymes (malate and glutamate dehydrogenases, lipases); they are important to protein and fat metabolism of the fungus, eliciting a decline or arrest of vital functions of the latter (G. T. Petrenko).

All of the submitted data are indicative of the desirability and prospects of research on antibiotic substances of higher plants. Since there are numerous antibiotics of microbial origin, which have and justifiably continue the "victory march" for a third of a century already in the field of public health and animal husbandry, what determines the desirability of such research? In the first place, it is promising because there are some distinctions in the plant antibiotics studied. It is generally conceded that there is a need to search for new antibiotics to treat bacterial infections induced by antibiotic-resistant strains of bacteria. On the basis of the experience gained with the use of substances, small in number thus far, it may be assumed that plant antibiotics (to which all antibiotic-resistant strains of staphylococcus were sensitive in our studies) not only have an antimicrobial

action in the organism, but enhance its defense capabilities, involving immune mechanisms in eradication of infection; they stimulate tissue regeneration, increase phagocytosis, normalize the composition of formed blood elements, increase immunogenesis and have anti-inflammation properties. Perhaps, these distinctions of the mechanism of therapeutic action of plant antibiotics explain, to some extent, the fact that resistance to these antibiotics of bacteria in the organism develops very seldom, even in the case of prolonged therapy, although it is possible to induce resistance to them in bacteria in vitro (V. A. Kudryavtsev, P. S. Volosovets). This distinction is of utmost importance to medical practice.

#### Investigation of Antiviral Properties of Plant Products

The acute need for antiviral agents in medicine, veterinary practice and plant growing prompted extensive research on synthetic compounds and substances of natural origin with antiviral properties. Nor were higher plants overlooked. But immeasurably more difficulties are involved in searching for antiviral agents than antimicrobial ones. The main difficulties are attributable to the distinctive interaction between viruses and sensitive cells, the metabolic processes of which are closely related.

Antiviral properties of plant preparations were studied in the department of antibiotics on the following models: *E. coli* bacteriophage (S. I. Zelepukha), tobacco mosaic virus, influenza virus in chick embryo tissues and experimental influenza infection of white mice, type III adenovirus (Ye. L. Mishenkova). Studies on the model of influenza virus were conducted primarily in conjunction with the Kiev Institute of Epidemiology, Microbiology and Parasitology (A. F. Frolov).

Studies were made of antiviral properties of various extracts, essential oils, fractions of acids, phenols, basic and neutral substances isolated from many plants, individual substances, antibiotics and analogues of natural plant antibiotics.

Some unadulterated juices and occasionally groups of substances from one plant presented phagocidal action when in contact with free extracellular bacteriophage (the juices of some food plants, ascorbic acid, gallic acid, the action of which is superior to the activity of many organic and inorganic acids, and certain other substances). But none of the preparations was active in experiments on reproducing bacteriophage.

In vitro and in vivo studies were pursued of TMV [tobacco mosaic virus]. Of all the extracts obtained from 250 plant species, only 15 depressed TMV in vitro (39-51% inhibition of necrosis) and in vivo (45-42%). Most active were preparations from *Statice latifolia*, essential oil from *Glycyrrhiza echinata* (44-70% inhibition) and *Psoralea drupacea* (70-90%); some alkaloids were found to be active, and the synthetic alkaloid, diuretin, was the most active. But the activity of all these substances was manifested only if leaves of indicator plants were treated prior to infection. Imanin was

found to be active against TMV, and this was established even earlier by A. D. Bobyr' (1959). Most of the active preparations inhibited not only development of the virus but accumulation of viral antigen to specific TMV antiserum.

Studies of antiviral action with reference to type III adenovirus were pursued on a culture of human amniotic epithelial cells. None of the extracts obtained from 130 tested plant species, including some that were active in experiments with bacteriophage and TMV, had an effect on type III adenovirus. Only 1 of 10 tested derivatives of thiosulfonic acid (allicin analogue) esters was active in a dosage that was not toxic to cells. This preparation (No 323) retarded the cytopathic action of adenovirus by 3 days.

There were 16 preparations, active on TMV, that retarded development of the cytopathic effect of type B influenza virus in a culture of chick fibroblast tissue and lowered its hemagglutinating and infectious titers, but activity was demonstrated in only 10 of them in doses that were nontoxic for cells: gallic acid, sodium gallate, tannin, preparation No 323, diuretin, a preparation from *Psoralea drupacea*, the antibiotics salvin, arenarin, helorgin and imanin. The same products were active (virucidal) with respect to type B influenza virus. Influenza A<sub>2</sub> virus was more resistant: it was inactivated only by gallic acid and sodium gallate used in nontoxic doses. In experiments on chick embryos infected with influenza virus (types B and A<sub>2</sub>), only gallic acid, imanin and preparation 323 lowered the hemagglutination and infection titers. The same agents, when tested on mice infected with 100 LD<sub>50</sub> type A influenza virus (strain PR-8), prolonged the life of 65-75% of the animals by 1-6 days when treated with gallic acid, 58-74% of the mice by 1-3 days in the case of imanin treatment and 51-55% of the animals treated with preparation No 323 by 1-3 days, but only if treatment was begun before infection. Retarded development of pathomorphological manifestations of influenzal pneumonia was observed in treated mice. Use of these agents in aerosol form was somewhat more effective than hypodermic injections.

Thus, the above products delayed development of influenza infection, accumulation of influenza virus (lowering hemagglutination titer) and retarded pathomorphological changes in the mouse lungs.

On the basis of subsequent investigations, it may be assumed that the antiviral properties of imanin, as well as the plant from which it was obtained (common St. John's wort), is probably attributable to the catechins and flavonoid glycosides they contain.

Analyzing the possible mechanism of antiviral action of plant products, we assumed that they may be inductors or stimulators of interferon production. One of the viral inhibitors isolated recently, preparation K, did indeed induce interferon production in a culture of chick embryo tissues, as was demonstrated with EEV virus used as an indicator test. There are also other, incomplected investigations indicative of stimulation of interferon production in the organism by some plant substances.



All of the laborious investigations that dealt with demonstration of antiviral properties of plants are indicative of the fact that plants can, apparently, serve as a source of antiviral agents.

#### Investigation of Antineoplastic Properties of Plant Products

In the department of antibiotics, much attention was devoted to the study of antineoplastic activity of plant products. We studied various products in collaboration with M. O. Shvayger, T. P. Mandrik, A. S. Bondarenko and L. S. Kunitskaya: antibiotics, alkaloids and extracts from 520 plant species. The preliminary stage of research involved the choice of methods for primary investigation of antineoplastic activity of preparations. The best results were obtained with the complex use of several methods of tentative evaluation of antineoplastic properties, which enabled us to detect active species for subsequent, more comprehensive investigation.

The tests were conducted using the solid form of Ehrlich's tumor and the ascitic form of sarcoma 37.

The group of active plants, the products of which inhibited growth of Ehrlich's tumor by over 40% (41-80%), was represented in our studies by 34 species referable to 16 families. The largest number of species (9 out of 34) was referable to the Compositae family, 3 were in the Rosaceae family, 3 in the Euphorbiaceae family and 2 each in the Ranunculaceae and Amaranthaceae families; the remaining 11 families were represented by 1 species. There was complete coincidence of the results of the primary in vitro tests using 3 methods simultaneously and in the experiments with transferable tumors in about 33% of the cases.

Some of the extracts and individual fractions of plants were tested as well on the ascites form of sarcoma 37. The highest inhibition level (81-100%) was demonstrated in products of two plant species; 71-80% delay in tumor growth was observed among products derived from 5 plant species, 61-70% was found in 4 and 51-60% retardation in products from 1 plant species. The products from other plant species were less active.

Sarcoma 37 is more sensitive than Ehrlich's tumor, and the indices of antineoplastic activity were higher for the former with most of the products tested. The only exception was referable to products of 3 plant species, the activity of which was equally expressed in both tumors, while products from 1 plant species had higher indices of inhibition of Ehrlich's tumor than sarcoma 37.

Mice tolerated well the hypodermic injection of products from most of the tested plants, used to treat both types of tumors. Only products from three plant species were highly toxic in the tested doses.

These studies enabled us to detect two plant species, referable to the Compositae and Leguminosae families, which were subsequently submitted to more comprehensive investigation with the use of other tumors, different doses,



different routes of administration, different times of starting treatment and different duration thereof (in multiple replicas).

Another group of plant products (numbering 380), obtained from plants referable to 38 families, was studied by D. G. Zatula, S. S. Krymovskaya, S. R. Reznik and A. S. Bondarenko (department of antineoplastic agents).

At first, all of the products were tested by various in vitro techniques. A total of 197 products were tested on animals with transferred tumors; 17 were found to be active; they were characterized by marked antineoplastic activity. The most active plants were referable to the Compositae, Labiatae, Leguminosae and Liliaceae families.

Two products (6 and 7), which merit further comprehensive investigation, were selected as a result of testing antineoplastic activity of all the preparations obtained in the department, which was performed by two teams of researchers. These products are complex; the active elements were isolated from both and submitted to investigation (A. S. Bondarenko). Preparation 6 is better studied with respect to biology, and it would be interesting to submit it to clinical investigation because of its high antineoplastic activity in some forms of malignant tumors of animals, low toxicity and lack of side-effects when given for a long time. For this reason, it was submitted to a more comprehensive study (D. G. Zatula, S. R. Reznik and others). These authors established the beneficial effect of this preparation on experimental brain tumors in mice; the therapeutic response was related to the number of cells inducing the morbid process, time when treatment was begun and its duration. Histological examination of various experimental tumors in mice treated with preparation 6 revealed a tendency toward regression, which was manifested by dystrophy of tumor cells, considerable necrosis, as well as a marked inflammatory reaction around the tumor with development of a granulation ridge and, in some cases, a tendency toward formation of a capsule (Yu. A. Barshteyn et al., Kiev Institute of Epidemiology, Microbiology and Parasitology).

Preparation 7 was also found to be promising, and its antineoplastic activity was confirmed (Ye. T. Ket'ko et al.).

Thus, the desirability of studying antineoplastic activity of plant products is unquestionable, for the purpose of finding antiblastomic agents.

The results of the studies described revealed that plants are a rich source of antibiotics with antibacterial, antidermatophytic, antiviral and antineoplastic action.

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VIRAL DISEASES OF PLANTS AND RESEARCH ON EFFECTIVE MEANS OF CONTROL THEREOF

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 2, 1978 pp 242-252

[Article by A. D. Bobyr', Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 25 Nov 77]

[Text] Research on plant viruses began at the Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy, UkAS [Ukrainian Academy of Sciences] in the early 1950's. At that time, studies were pursued primarily of the viruses of clover mosaic, yellow disease of sugar beets, as well as to find inhibitors capable of suppressing the pathogens of viral diseases of plants (B. P. Matsulevich, A. D. Bobyr', V. A. Goryushin, A. A. Skofenko and others).

Research on viral diseases of agricultural plants assumed a wide scope in the Ukraine in 1960, when a department of plant viruses, headed by S. N. Moskovets, doctor of agricultural sciences, was created at the institute. This department conducted enormous amounts of work dealing with viral diseases of agricultural plants in this republic (V. G. Krayev, A. L. Boyko, L. G. Bilyk, L. Ye. Glushak, D. P. Grama, A. N. Onishchenko and others); much attention was devoted to development and refinement of methods of controlling them (A. D. Bobyr', L. K. Zherebchuk, M. I. Mendzhul, I. P. Zhuk, V. M. Anokhin, A. G. Kovalenko and others).

At the first stage of research in the department, studies were made of the main viral diseases of the most important agricultural plants in the Ukraine and other Union republics, their distribution and harmfulness, detection of diseases and identification of their pathogens, as well as circulation of the latter in nature. In the 15 years of existence of the department of plant viruses, studies have been conducted of such diseases as striped mosaic and mosaic of winter wheat, mosaic of corn, lucerne, lupin, beans used as feed, watermelons, cantaloupe, yellow disease of sugar beets, ridged mosaic of potatoes and soybeans, "gotik" [?] of potatoes, viral chlorosis and intervein mosaic of hops, perivein mosaic of clover, common mosaic of beans, common and green mosaic of cucumbers, mosaic and big bud of tomatoes, pepper and eggplant [1-3].

A search is being pursued at the institute since 1955 for antibiotics and other substances capable of depressing phytopathogenic viruses, as well as studies of their antiviral action. As a result of considerable work, about 700 various substances have been tested for antiviral action and over 165 inhibitors have been found among them, which depress tobacco mosaic virus (TMV) in vitro and in plants of the Solanum family, concurrently with 4-7 of the most deleterious viruses: TMV, the virus of cucumber mosaic (VOM-1), tomato bronzing virus (TBV), X, Y, S and M viruses of potatoes by 33-75% or more [4, 5].

To date, many various chemical and biological substances, as well as physical factors, that inactivate TMV and other phytopathogenic viruses markedly have been discovered. However, they did not find wide application in practice for therapeutic purposes. The obstacle to practical use of inhibitors is referable primarily to the fact that viruses are so closely associated with the host plant cell in their existence that, to reproduce, they utilize the systems and energy resources of this cell. For this reason, the absolute majority of known inhibitors of viruses is toxic to plants, by depressing viruses they also depress functional processes of the cell itself. Inactivation of virus, induced by an inhibitor used in a low concentration, at which it manifests relatively mild phytotoxicity, is partially or completely reversible. With regard to most of the known antiviral agents, with increase in concentration thereof, time of contact with the object and frequency of treatment, there is a significant increase in degree of viral inactivation and decrease or disappearance of reversibility of inactivation.

The antiviral action of the absolute majority of the inhibitors we found is nonspecific: as they depress the virus in the plant, they also depress the plant itself severely. However, their phytotoxicity is not solely related to species and varietal specifics of a plant, but presence of viral infection in it, as well as method of using the inhibitor. As a rule, the phytotoxicity of inhibitors is considerably lower in healthy plants than sick ones. The sharp increase in phytotoxicity of some inhibitors, against the background of infection, is attributable to simple summation of the deleterious effect of the inhibitor and viral infection and mutual enhancement of the toxicity of each of them. We find that the two factors, toxicity of the inhibitor to the host plant and reversibility of its inactivating effect, are related, in a diametrically opposite way, to the concentration of antiviral substance and duration of its effect on the virus and plant, and they are the main obstacle to practical use of viral inhibitors. They render ineffective and unpromising the treatment of sick plants with biological and chemical antiviral agents.

The data we obtained from testing a large number of various inhibitors of phytopathogenic viruses indicate that, when given preventively to healthy plants, where the toxicity of antiviral agents is much milder than in sick plants, they offer real possibilities for successful use of many active inhibitors with nonspecific antiviral action. However, research dealing with finding and screening antiviral agents for practical purposes is empirical

in most cases. We have demonstrated some general principles, on the basis of studies of antiviral action of a number of various chemical and biological substances, which may be used to solve problems of chemoprophylaxis and therapy of viral diseases of plants.

When searching for antiviral agents and screening them for practical use, one should proceed, first of all, from the thesis that chemoprophylaxis and therapy of viral diseases of plants cannot be resolved apart from biochemical, physiological and other processes in the plant, which determine viral reproduction in it and toxicity of the inhibitor for the host plant. For this reason, antiviral agents should be screened according to degree of toxicity, not only to the virus but the plant, i.e., one must take into consideration the species and varietal specifics of each host plant, as well as the stages of development at which it is stricken the most severely by specific diseases and responds to the inhibitor. It should also be borne in mind that the inhibitor must not have a stimulating effect on any stage of development of viral infection in the plant.

The possibility of recovery of depressed viral infectivity raises the question of the need to check inactivation of the virus by inhibitors in each instance of use thereof. In screening antiviral agents according to extent of irreversibility of viral inactivation, the following must be determined: concentrations corresponding not only to maximum antiviral activity in the absence of a toxic effect on the plant, but total irreversibility of viral inactivation; concentrations that have a high antiviral activity with negligible phytotoxicity and partial reversibility of viral inactivation, provided the latter can be prevented or diminished in the plant by extending inhibitor treatment time.

Most agricultural plants are more often stricken by several or even many very deleterious viruses, rather than a specific one, so that the efficacy of highly active inhibitors, which affect only a specific virus, diminishes sharply. The harm inflicted to the plant by this virus, when it is neutralized by an inhibitor, can be easily compensated by other viruses that strike it severely.

In order to enhance the reliability of protecting agricultural plants against viral diseases by means of chemical and biological agents, one must search for and screen inhibitors as well according to the spectrum of antiviral action covering the entire range of the most harmful viruses for each individual host plant. This task can be done, not only by searching for inhibitors with a wide spectrum of antiviral action, but by possibly combining two or even several inhibitors.

Adherence to these general principles will make it possible to avoid considerably the empiricism that prevails in the search and screening of antiviral agents for practical purposes, and will render work in the field of chemoprophylaxis and therapy of viral diseases of plants more purposeful and productive.

On the basis of the quantity and level of activity of inhibitors found in the various tested groups of agents, it can be concluded that antibiotics and other products of plant and microbial origin are the most promising for the search of highly active inhibitors capable of depressing viruses in plants. The nature and level of antiviral action of all inhibitors we studied enabled us to divide them into three large groups: 1) inhibitors with high antiviral activity in vitro and in vivo; 2) inhibitors with high antiviral activity in vitro, and 3) inhibitors with high antiviral activity in vivo. The in vivo antiviral activity of inhibitors in the second group and in vitro activity of those in the third group is relatively mild or absent.

The group of inhibitors with high antiviral activity in vitro and in vivo offers the most realistic possibilities and prospects for practical use in the control of viral diseases of plants. The results obtained from studies of many various inhibitors in field experiments indicate that some of them (metabolic products of some yeast of the genera *Saccharomyces* and *Candida*, bacteria of the genus *Pseudomonas*, as well as the antibiotics, imanin and arenarin) provide for an appreciable increase in harvest, when used preventively against the viruses of tobacco and ridged mosaic of potatoes, tobacco and tomatoes by spraying the superficial part of the plants or treating seedlings of these plants with solutions of the tested inhibitors [6].

Some inhibitors, which have both a direct effect on viruses and through the plant, can be used with success for preventive purposes by means of pre-planting treatment of seeds and tubers, in which their phytotoxicity is manifested much less than in young, vegetating plants. We refer to the metabolic products of the yeast and bacteria just mentioned, which augment appreciably the harvest of Priyekul'skiy early potatoes, not only in the case of 5-fold spraying of young plants in the field, but presoaking of tubers. The antibiotic, arenarin, metabolic products of some yeasts and fungi, when used to presoak tomato and pepper seeds, increase appreciably the harvests from these crops.

Inhibitors of phytopathogenic viruses that have a stimulating effect on the plant are of particular interest for practical purposes.

At the present time, two such antibiotics are being introduced in agricultural practice; they were obtained at the Institute of Microbiology and Virology, UkAS, from common St. John's wort and *immortelle*. Preventive use of these antibiotics on tobacco, tomatoes and potatoes, both in the form of pretreatment of seeds and tubers, and spraying healthy seedlings or young plants, increases plant resistance to viral infection. In tomatoes and tobacco, not only do these antibiotics manifest a preventive, antiviral action, but appreciably stimulate plant growth and development.

Preventive use of imanin on tomatoes, in the closed ground, prevents shedding of ovaries and increases fruit formation, as well as appreciably increases carbohydrate content in the fruit. In tobacco, imanin causes

development of sturdier seedlings, as a result of which it holds better in the soil, especially in the dry climate of Crimea. There is a significant increase in carbohydrate content of tobacco leaves and, conversely, a decrease in protein content, which has a beneficial effect on the quality of tobacco raw material and products.

Many years of industrial and state testing of the preventive use of imanin on tomatoes and tobacco at experimental centers, kolkhozes and sovkhoses of the Ukraine, Abkhazia, Moldavia and other republics offer convincing evidence of the high efficacy of this treatment. Five-fold spraying of tomato and tobacco seedlings prior to transplanting in the ground, using a 0.01-0.05% imanin solution, reduces TMV and tomato bronzing by 50-75%; this also reduces by over 2-3 times the number of plants stricken by bronzing, as a result of which the harvest is 15-20% higher. The income resulting from such treatment constitutes 75 kopeks to 1 ruble per m<sup>2</sup> for tomatoes in closed ground, and up to 300 or more rubles per hectare for tobacco. All of the expenses for such treatment of plant seedlings with antibiotics constitute less than 1% of the cost of the added harvest.

Imanin is highly effective when used preventively against TMV, TBV and ridged mosaic of potatoes, not only on tobacco, potatoes and tomatoes, but pepper and eggplant. Imanin has an analogous effect against VOM-1 in cucumbers, scallop squash, vegetable marrow and other Cucurbitaceae, as well as against mosaic virus in corn, increasing the harvests from these plants appreciably [3-5, 7].

More than 20 preparations were obtained from filtrates of culture fluid and biomass of yeasts of the genera *Candida* and *Saccharomyces*, which depress strongly the infectivity of TMV, X virus of potatoes, VOM-1 and certain other viruses. The most active ones were forwarded to the Ukrainian Scientific Research Institute of Agricultural Microbiology, Southern Department of the All-Union Agricultural Academy imeni Lenin, for further study to enhance the efficacy of meristem cultures in protecting potatoes against viruses.

Healthy planting material is very important in the control of viral diseases of potatoes. Different routes have been tried to solve this problem. At one time, summer planting of potatoes was used for this purpose, but it was ineffective; moreover, the potatoes used for planting were imported from northern regions to southern ones, or from the mountains to the lowlands. However, potatoes are also stricken by viruses in the north, and transporting them over long distances is hardly warranted economically. The climate of mountain regions help safeguard potatoes from viral diseases and improve its seed qualities, but this effective method of ameliorating planting material is of regional significance for the mountains.

As for the southern part of the republic, where viral diseases of potatoes are the most widespread, the most promising practice is to plant potatoes in the summer using freshly gathered tubers and irrigation. The dormancy period of freshly gathered tubers is eliminated by treating them with the

stimulators, gibberelin and thiourea (presoaking for 30 min in a mixture of 0.0001% gibberellin and 2% thiourea), and this provides for normal sprouting of potatoes.

Considerably less invasion of plants by viruses is obtained when potatoes are planted in the summer, using freshly gathered tumors treated with gibberellin and thiourea, than in fields where they are planted at the usual time, in the spring. And if selection is additionally made of plants with healthy external appearance, the new crop of tubers are essentially healthy and can be used as planting material. Early summer planting of potatoes using freshly gathered tubers treated with gibberellin and thiourea aids in obtaining healthier planting material, free of viruses, and makes it possible to obtain two harvests of potatoes per year, with irrigation [2-8].

Inhibitors with superhigh phytotoxicity are also of some interest to practice. We have already mentioned that there is manifestation of different extents of toxicity by these inhibitors when used on healthy and sick plants: they inflict negligible harm to healthy ones, whereas they affect sick ones similarly to herbicides, causing tissues, leaves and even the entire plant to die. The use of such inhibitors on seedlings not only increases plant resistance to disease, but kills (in the case of infection) sick plants or discloses them as latent virus carriers. As a result of such viral "weeding" or early detection of plants that are latent virus carriers, the sick seedlings would not be transplanted in the field or hothouse, where they could serve as the source of widespread infection. The use of such inhibitors is of particularly great interest on plants that are grown by means of seedlings. We found several cultures of fungi and yeast, the metabolic products of which influence tobacco plants in the presence of viral infection in the same manner as herbicides; simazin, atrazine and several other physiologically active substances in the triazine group have analogous action [9].

Broader use in practice of highly active antiviral agents that present high phytotoxicity also merits attention for disinfection purposes. On the basis of data in the literature and our own findings, to decontaminate agricultural objects and the soil from viruses, one can use caustic alkalis, quick lime, bentonite, trisodium phosphate, potassium manganate, skimmed cow's milk and whey; the last three substances can be used to disinfect the hands of workers caring for healthy and sick plants in hothouses, nurseries and in the field. A number of highly active inhibitors with nonspecific antiviral action can be used to attenuate the infectiousness of very harmful phytopathogenic viruses [10]. By using such attenuated viruses to inoculate plants, one can protect them partially or completely against infection by the very virulent forms that cause enormous detriment to agricultural production. From this point of view, of interest are also such potent physical factors as gamma radiation, ultrasound and steady magnetic fields, which are capable of inducing significant changes in biological, antigenic and other properties of some phytopathogenic viruses that strike hops and essential-oil-bearing roses [11].

The combined use of radiation, heat therapy, antiviral agents and trace elements yields beneficial results with hops and roses. The results obtained are indicative of the fact that, using these factors in a special heat chamber, one can obtain virus-free apices in vegetating plants [12].

At the present time, mineral and organic fertilizers are gaining special importance in augmenting the harvest of agricultural plants. As we know, soil, peat, humus, plant residue, which often contain viruses that strike agricultural plants, are often used to prepare organic and mineral mixtures and composts. Some of these viruses, primarily TMV and VOM-1, retain infectiousness even after the composts ripen, so that they should be decontaminated. As shown by the experience of some foreign researchers and our own data [13], addition to the base compost material of a certain amount of soil from wheat, rye, pea and flax fields, as well as forest ground cover, fallen cherry, apple, pointed-leaf linden, elm, ash leaves, as well as leaves that are not suitable for silage from sugar, feed and table beets, the juice of the roots and leaves of which contains potent inhibitors of many very harmful plant viruses, inactivates viruses contained in ripening composts. Wide use of antiviral substances contained in various plant residues in agriculture for preventive purposes in preparing composts would prevent further spread of serious viral diseases of agricultural plants.

It was recently shown that addition in plant tissue of heterologous RNA and synthetic analogues thereof induces development of resistance to viral infection. A virus isolated from the mycelium of *Penicillium funiculosum* fungus and its nucleic acid [14], as well as certain other substances, have the capacity to induce resistance to some phytopathogenic viruses. Studies dealing with the possible use of inductors of plant resistance to phytopathogenic viruses are quite promising and merit special attention.

In our opinion, these are the realistic possibilities and principal routes for extensive practical use of some already known inhibitors of phytopathogenic viruses.

Thus, in the 15 years of existence of the department of plant viruses at the Institute of Microbiology and Virology UkAS, its staff has defined the phytovirological situation in a vast natural area, the Ukraine: an index has been made, with determination of presence and incidence of the main viral diseases, of the principal agricultural plants in the republic and their economic significance. Serological, electron microscopic and other methods have been defined and developed for diagnosing most of the studied diseases of plants and identifying their pathogens; the routes and means of circulation of the latter in nature have been determined. Many highly active inhibitors of diverse phytopathogenic viruses have been found. There has been demonstration and experimental substantiation of the correlation between a number of factors determining the effectiveness of antiviral agents and their toxic effects on viruses and host plants. On this basis, general principles have been elaborated for finding and screening



the most effective inhibitors for practical use; the ways and means of broad use thereof have been outlined for the control of phytopathogenic viruses. Recommendations have been prepared and forwarded to the Ukrainian Ministry of Agriculture, on the basis of which scientifically substantiated, effective measures can now be applied to control viral diseases of potatoes, sugar beets, tobacco, tomatoes, hops, leguminous and other agricultural crops (Tables 1 and 2).

Table 1. Effectiveness of practical recommendations on the control of viral diseases of plants

Recommendations	Reduction in sick plants, % control				
	ridged mosaic of potato	striped mosaic of wheat	yellow disease of sug. beet	common mosaic of beans	big bud
Denser sowing or planting	—	—	80	30	60
Optimum sowing time	—	90	60	26	78
Extermination of virus-carrying insects	—	—	70	50	75
Use of freshly gathered tubers for potato planting	75	—	—	—	—
Spatial isolation	—	—	90	—	—
Planting potatoes in the mountains and closed areas	85	—	—	—	—
Nontransplanted crops	—	—	—	—	94

Note: —) not studied.

Table 2. Economic effectiveness of preventive use of imanin, gibberelin and  $\gamma$ -radiation against tobacco, ridged and intervein mosaic, bronzing and chlorosis of tobacco, tomato, potato and hops

Product used	Method of application	Decline of		Harvest increm c/ha % control	Economic effectiveness (net income)
		virus titer, %	number of sick plants		
Antibiotic, imanin	Five-fold spraying of seedlings with 0.01% solution before transplanting in the ground	50—75	by 2—3 times	1,5—3,0 15—20	0.7—1,2 ruble per m <sup>2</sup> tomatoes; 300—500 rubles/ha tobacco
Stimulators, gibberellin and thiourea	Presoaking tubers in 0.0001% thiourea	—	60—70%	100—150 80—100	400—600 rubles/ha potatoes
$\gamma$ -Radiation	Preirradiation of hops cuttings, 100 rad dosage	50—70	—	3—4,3 22—32	over 500 rubles/ha hops

Key: c) centner

The following are general measures for the control of viral diseases of plants:

1. Development and introduction in agricultural practice of plant varieties that are resistant and immune to viruses. Use of resistant varieties that also have other properties useful to man is the most effective and reliable means of controlling viral diseases.
2. Extermination of insects and other carriers of viruses. Virtually all viruses, with some exceptions, are transmitted from sick to healthy plants by various insects, aphids, thrips, cicadas, ticks, and even the parasite plant, the dodder.
3. Eradication of natural sources of viruses. This refers, first of all, to removal of weeds from the fields, road shoulders and sides [boundaries], which are reservoirs of viruses in many cases, and additionally, viruses can overwinter in perennial weeds.
4. Raising and using healthy, virus-free seeds for sowing. Many viruses are transmitted by seeds, tubers, cuttings and other planting material; in order to have such healthy planting material, it should be prepared on plantings that are not stricken by viral diseases or with a minimum number of sick plants.
5. Finding and introducing in agricultural practice antibiotics and other biological and chemical agents capable of depressing infectivity of phytopathogenic viruses would create the conditions for implementing direct preventive measures against the most harmful viruses that cause enormous detriment to the yield of agricultural crops.
6. Use of agrotechnical measures that reduce the incidence of viral diseases in agricultural crops. They include the following: a) avoiding sparse sowing and planting; b) adherence to optimum density of plantings as stipulated for different crops in the agricultural rules; c) abiding by optimum times for sowing and planting, as early as possible; d) instituting irrigation of cultivated crops where feasible; e) adhering to proper crop rotation, which would rule out perennial cultivation of a given crop on the same field or precursors that are stricken by the same diseases; f) maintaining spatial isolation from crops that have the same disease and from infected areas of the previous year; g) raising plants on an optimum prepared soil with adherence to proper watering schedule, without excessive soaking of the soil, reduces the spread of disease; unilateral application of excessive doses of nitrogen fertilizer lowers plant resistance to viral diseases, while the use of potassium fertilizer, on the contrary, increases it.

The study of the principal viral diseases of plants and development of measures for the control thereof in such a vast natural geographic region as the Ukraine constitutes the first attempt at such an investigation, and it should be continued and refined. But, highly sophisticated agricultural practices are unquestionably the most important factor in the control of

viruses and viral diseases of plants, i.e., an aggregate of measures that would assure raising healthy plants, with constant growth of harvests of cultivated crops and, at the same time, aid in increasing soil fertility.

At the second stage of the research, begun in the 1970's, a broad front of studies is being conducted in the department of plant viruses (in 1974, the department of plant virus reproduction, department of phytopathogenic viruses and unstructured laboratory of algal viruses were created on its basis) of morphology and fine structure, physicochemical, antigenic and other properties of plant viruses, blue-green algae and microscopic fungi, their relations to the host cell, localization and reproduction in the plant, physiology and biochemistry of plants stricken by viruses, plant resistance to viruses and resistance of the latter to various environmental factors, etc.

In particular, research on antiviral products from yeast is being conducted in the department of phytopathogenic viruses: their polysaccharide nature has been established; physicochemical properties and biological activity with regard to a number of phytopathogenic viruses have been studied [15]. Investigations are in progress of the effects of various physical factors on viruses that strike perennial plants. Exposure of TMV isolated from essential-oil-bearing roses to different doses of  $\gamma$ -radiation elicits appreciable changes in biological and serological titer of this strain. Delivery of high doses of radiation to viral suspensions elicits denaturation and coagulation of the viral protein membrane and, apparently, partial breakdown of antigenic groups of viral protein [11]. Almost analogous changes are also observed in viruses that strike hops. When elementary particles of hops leaf curl virus are exposed to radiation, they accumulate in part on specific sites or segregate into smaller fragments; a decline of titer of antiserum obtained to irradiated antigen is observed. There is an appreciable change in antigenic activity after irradiation in the virus of inter-vein mosaic, which has spherical elementary particles.

Exposure of viral preparations of TMV to ultrasound and steady magnetic field (SMF) results in significant loss of infectiousness and breakdown of elementary viral particles (virions) into smaller fragments. There are particularly appreciable changes in morphology of the virus after delivery of high doses of ultrasound and SMF, with change in other viral properties as well.

A study of the nature of genetically determined plant resistance to viruses revealed that disruption of the process of transcription and translation by selective inhibitors (actinomycin D, rifomycin, cycloheximide, chloramphenicol) does not elicit induction of infectious virus synthesis in the immune cell. These data are indicative of insensitivity of the viral inactivation system to the factors studied, or anomalous interaction between the virus and immune plant (Kovalenko, Boyko and others).

Research is being pursued in the department on changes in cell metabolism of plants with different types of viral resistance (A. G. Kovalenko, L. K. Zherebchuk and others). It was shown that, after using KhVK [X or chlorosis

virus of potatoes?] to inoculate potatoes that are extremely resistant to this virus, virtually no end infectious products of viral synthesis (nucleo-protein, RNA or unstable forms of the virus) are demonstrable in plant tissues. It was found that KhVK can induce changes in metabolism of inoculated plants presenting no outward signs of disease, and this is of interest to the study of mechanisms of plant resistance to the virus. Development of acquired resistance and necrotization of cells, which occur simultaneously in inoculated plants, present no overt correlation common to various forms of tobacco. This warrants the assumption that these two processes are controlled by several different genetic factors, which interact independently of one another with the products of expression of the viral genome in plant cells.

It was shown that, in the course of the infectious process, there are minimal fluctuations of nucleic acid content in virus-resistant varieties and considerable fluctuations, in susceptible especially during the period of intensive reproduction. It was found that elevation of nuclease content in immune plants after inoculation with virus is one of the factors of cell immunity to viral infection.

Methods have been developed for cultivating, isolating and purifying viruses that strike fungi of the genus *Penicillium*; their physicochemical, biological, antigenic, inhibitory and inductor properties have been studied. It was shown that plant resistance to viruses, induced by the virus of *Penicillium funiculosum* and its RNA, as well as the double-helical complex of polyguanylic and polycytidylic acids, is sensitive to actinomycin D, which is indicative of the need for DNA-dependent synthesis of RNA in the plant cell in development of this type of resistance to viruses [14, 16].

The fact that these agents can induce resistance to phytopathogenic viruses in plants opens up new prospects and great opportunities for speedy development and scientific substantiation of theory of artificially acquired immunity and extensive use of the latter in the control of viral diseases of agricultural crops.

Conferences, meetings and congresses have been very important to the development of virological research at the Institute of Microbiology and Virology, UkAS. The staff of the department of plant viruses organized the 5th and 6th All-Union conferences on viruses and viral diseases of plants, which convened in Kiev in 1964 and 1971. At these conferences, more than 280 scientific papers were delivered, which is almost twice the number delivered at the 4 preceding conferences; of this number, 50 papers were authored by the staff of the department of plant viruses. The proceedings of these meetings were published in Kiev, by the Naukova Dumka Publishing House, in 1966 and 1974. In addition, members of the staff of this department have published more than 10 monographs and booklets dealing with phytovirology. The Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy, Ukrainian Academy of Sciences, is justifiably recognized as one of the major centers for phytovirological research, not only in our country but abroad.

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STRUCTURAL COMPONENTS OF ADENOVIRUSES AND CERTAIN OTHER DNA-GENOME VIRUSES

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[Article by N. S. Dyachenko, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 28 Nov 77]

[Text] Comprehensive structural and functional investigation of virion components, in particular, capsid proteins, including definition of physical parameters and antigenic specificity of different proteins, their functional role in expression of the viral genome and composition (morphopoiesis) of the virion, and determination of the mechanisms of immunoinactivation of virions constitute one of the intensively developing directions of modern virology. To this problem are linked some questions that are related to investigation of the effects of viruses and their components on different elements of the immunocompetent system, which is important to comprehension of the mechanisms of long-term persistence of some viruses in the macro-organism and pathogenesis of the diseases they induce. By virtue of a number of biological and structural distinctions, adenoviruses are a convenient object for experimental investigation of the above-mentioned problems. The present work deals with analysis of the data we have obtained in this direction.

The proteins that make up the adenoviral capsid (hexon, penton base and fiber) differ in localization, shape and size of molecule, primary structure and antigenic specificity [1, 2], which makes it possible to isolate them and study them, using for this purpose the entire armamentarium of modern methods of protein chemistry, immunology, etc.

Comprehensive investigation of structural proteins of adenoviruses is part of a many-year project of the department of molecular biology of viruses at the Institute of Microbiology and Virology, UkAS [Ukrainian Academy of Sciences], dealing with adenoviruses and processes they induce in the cell during reproduction. These studies were begun in 1968 and are still in progress (N. S. Dyachenko, L. N. Nosach, N. P. Vantsak, E. G. Mikhaylova, G. G. Popenko, L. V. Tokarchuk, N. Ya. Spivak, A. D. Shved, S. K. Votseiko, V. G. Lisovenko, K. P. Gushcha, V. L. Zhovnovataya and N. A. Mudrik).

The main objects of the studies were adenoviruses and, in some experiments, phages of phytopathogenic *Erwinia* bacteria as a convenient source of obtaining virus-specific macromolecules, in particular DNA. A number of methods was used: electron microscopy, sedimentation analysis, in particular centrifugation in a saccharose and cesium chloride density gradient, electrophoresis in polyacrylamide gel, ion-exchange chromatography and gel filtration, immunofluorescence and cytofluorometry, as well as diverse serological reactions, including the passive hemagglutination reaction that we modified.

We devoted much attention to the comparative study of molecular properties of capsid proteins of types 1 and 10 adenoviruses (AB), representing Rosen's third and second groups, and differing sharply in hemmagglutination properties. It should be indicated that there are only isolated works dealing with type 1 AB [3, 4], and no studies have been made in this direction at all of type 10 AB.

We used ion exchange chromatography on columns filled with DEAE-sephadex A-50 to obtain capsid proteins. The projection, penton and hexon of type 1 AB are eluted in 0.025-0.05 M, 0.15-0.175 M, 0.425-0.475 M or 0.475-0.5 M sodium chloride solutions. The projection [or penton fiber] of type 10 AB is desorbed with 0.025-0.075 M NaCl and hexon, with 0.475 M NaCl. A soluble virus-specific protein is eluted among proteins of type 10 AB with 0.225-0.275 M NaCl, which is demonstrable in the reaction of direct agglutination of rat erythrocytes as a complete hemagglutinin [5, 7]. Thus, a similarity is found in chromatographic profiles of projections and hexons of types 1 and 10 AB when separated on columns with ion exchangers. Analogous results were obtained after gel filtration of different types of AB hexons on columns with agarose 4B [8].

Capsid proteins are partially purified after one cycle of chromatography. More complete removal of admixtures was obtained by repeated chromatography and centrifugation in a saccharose density gradient. It was found to be desirable to use an immunosorbent of serum against the principal contaminant proteins to purify projections of type 1 AB. The homogeneity of the obtained preparations of adenoviral proteins was established on the basis of electron microscopic, sedimentation, immunoelectrophoretic and serological examination.

According to electron microscopy findings, AB projections of both types are rod-shaped elements situated on the apices of the icosahedral capsid, and they have a head on the end. They are visualized more often and more distinctly in AB type 10 virions than in type 1. The length of the projections of type 10 AB constitutes  $125 \pm 11$  Å, the width is 35 Å and the diameter of the head is 45 Å; projections are not encountered in a free state. In preparations of AB type 1, there are both isolated projections and some joined to the apical capsomeres (pentons). The overall length of a penton is  $377 \pm 21$  Å, the length of the projection is  $297 \pm 26$  Å, the diameter of the latter is 25 Å and the diameter of its head is about 45 Å. Consequently, the sizes of projections of types 1 and 10 AB differ significantly, and projections of the former are almost 3 times longer.



Aggregates of 12 pentons, named dodecons, are demonstrable in preparations of type 10 AB. Their structure is consistent with the principles of icosahedral symmetry; the overall diameter is 470 Å and core diameter is 235 Å. Dodecons are a complete hemagglutinin and are demonstrable in subgroups I and II of AB, according to the classification of Rosen [2].

Hexons of both AB types have the shape of polygonal prisms  $86 \pm 4$  Å in size, with a 25 Å central core [canal] [9-11].

Determination of the sedimentation coefficients and molecular weight of AB types 1 and 10 capsid proteins by the method of Martin and Ames (1961) revealed that the projection of type 1 AB has a sedimentation coefficient of  $6.3 \pm 0.2$  S and molecular weight of  $98,000 \pm 4000$  dalton, and that of type 10,  $4.9 \pm 0.2$  S and  $76,000 \pm 5100$  dalton, respectively. The difference between these indices is statistically reliable ( $p < 1\%$ ). Hexons present virtually no differences in these parameters: the coefficients of sedimentation and molecular weights constitute 11.4 S and 260,000 dalton; 11.1 S and 254,000 dalton [12]. Direct sedimentation analysis of AB type 1 hexon revealed that its sedimentation constant,  $S_{20,w}^0$ , equals 12.4 S [13]. Consequently, the molecule of the projection of type 1 AB is considerably larger than that of type 10 AB projection.

The hypothesis has been expounded that this difference should be related to the difference in complexity of antigenic structure of the two proteins. In type 1 AB, we found a new sensitizing antigen, which is tested for capacity to adsorb on tannin-treated bovine erythrocytes, as a result of which the erythrocytes are agglutinated by specific serum [14, 15]. In addition, the activity of incomplete hemagglutinin is associated with the projection and penton of type 1 AB, like other representatives of Rosen's subgroup III. It is demonstrable by the capacity to be adsorbed on rat erythrocytes, which is then agglutinated by specific antiadenoviral serum [2, 3].

Several experiments dealt with the properties of sensitizing (CE) antigen of AB type 1 and demonstration of its activity in preparations of type 10 AB proteins. It was established that adsorption of CE antigen is more intensive at a temperature of 4°C than 37°C, and elution is demonstrated at 37°C. CE antigen is resistant to trypsin, so that it is unlikely that it is associated with the trypsin-sensitive penton base [16]. CE antigen resembles penton fiber with respect to heat sensitivity; its chromatographic profile is similar to that of the projection and incomplete hemagglutinin; it is demonstrable in preparations of purified projection, i.e., the analyzed material is indicative of the fact that CE antigen is contained in the projection. It contains a type-specific component, which reacts with erythrocytes and is situated in the distal part of the molecule, as well as a component in common with members of subgroup III, which is localized in its proximal part and interacts with antibodies. The type-specific components of CE antigen and incomplete hemagglutinin differ, since injury of the former after adsorption on tannin-treated erythrocytes does not affect the activity

of the latter. A series of experiments dealt with demonstration of activity of CE antigen in a projection of type 10 AB and it failed to yield positive results.

Thus, a comparative study of projections of AB types 1 and 10 established the more complex antigenic structure of projections of AB type 1 and presence of CE antigen in it, i.e., the larger size of the projection molecule of AB type 1 corresponds to more complex antigenic composition, which is also consistent with its greater functional activity.

In the past few years, there has been development of the conception of complex antigenic structure of adenoviral hexons [1, 18, 19]. The group-specific determinant of hexons is readily detectable in the reaction of immunodiffusion in gel or CFR [complement fixation reaction] in monomeric hexons. We examined the nature of interaction between particles of type 1 AB with sera against hexons of AB types 1 and 10 to demonstrate the type-specific determinant. The latter is localized on the external surface of the capsid, since it was demonstrated by electron microscopy that antibodies only against homologous hexon are attached to virions. Around the virions, a layer of antibodies is formed, which is about 125 Å in thickness; the capsid structure is not distinctly visualized, and occasionally there is formation of small aggregates of virions. Upon contact with antiserum to hexon AB type 10, antibody-free virions are seen with clearly visible capsid structure. This indicates that the group-specific determinant of hexons is not demonstrable within the virion. Probably it is changed into the capsid cavity [9, 10, 13].

The group-specific determinant may be classified as cryptotopic, i.e., undemonstrable in this position in the capsid, and the type-specific determinant can be classified as metatopic, i.e., on the external surface and functioning as part of the virion [18, 20].

The intracellular localization of specific hexon determinants has been investigated. For this purpose, a comparison was made of nature of fluorescence in cells infected with different types of adenoviruses and treated with serum to homologous and heterologous hexons (L. N. Nosach, N. S. Dyachenko and others).

We conducted a series of experiments to study the neutralizing activity of anti-hexon antibodies, since the mechanism of neutralization of infectiousness of adenoviruses is still unclear. According to traditional conceptions, neutralization of infectiousness occurs as a result of blocking by antibodies of the  $\gamma$ -determinant of projections of the adenovirion, with which it is adsorbed on the cell. However, Shortridge [21] believed that neutralization could also occur upon interaction between antibodies and hexons surrounding the apical capsomeres with projections (peptons), by means of steric overlapping and masking of projections by the antibodies. Yet this is unlikely to apply to AB of Rosen's subgroup III, in view of the considerable length of their projections. Moreover, several authors [1, 22] have

demonstrated that antihexon antibodies are capable of neutralizing the infectiousness of some AB types. For this reason, it was deemed interesting to demonstrate the mechanism of immunoactivation of AB type 1, which is referable to the subgroup with the longest and antigenically more complex projections, as well as to determine the specificity of the antigenic determinant involved in this process. The hexon determinant to be neutralized must be in the metatopic position on the surface of the adenovirion.

It was established that antibodies to hexon neutralize infectiousness of adenoviruses, of both the homologous and heterologous types. However, the reaction is much more intensive in a homologous system than heterologous. Thus, the constant of rate of neutralization of type 1 AB by antibodies to homologous hexon is 56.3, and by antibodies to hexon of types 2 and 10 it is 0.63 and 1.55, respectively (in all cases, incubation time was 60 min). In our opinion, these data confirm, once more, the hypothesis that there is no group-specific hexon determinant on the outer surface of the adenovirion, since in such a case the neutralization process should have occurred at the same intensity in both systems and the constants of neutralization rate would be similar (N. S. Dyachenko, L. V. Tokarchuk, L. N. Nosach).

Much attention was devoted to the study of distinctions of localization and dynamics of accumulation in the cell of early and capsid proteins of type 1 AB, for which purpose we used sera that we had prepared to early (P) antigen and different capsid proteins in the indirect method of fluorescent antibodies. It was established that P antigen is demonstrable 6 h after infection in 4.5% of the cells, in the form of fine fluorescent dots in the nucleus; in some cells diffuse fluorescence of the perinuclear cytoplasmic region is also observed. The maximum number of cells with accumulations of P antigen (32.4%) and maximum number of granules of this protein are observed 11-12 h after infection, after which these indices drop [23].

Hexon antigen is first demonstrable 14-15 h after infection in the form of fluorescent granules scattered in the nucleus and diffuse fluorescence of cytoplasm. After 2-3 h, in some cells it is distributed in the form of fine-grain inclusions and continues to be demonstrable in the form of diffuse fluorescence of the perinuclear region. Later on, as the inclusions become consolidated, there is formation of additional accumulations of hexon antigen under the envelope of the nucleus, and the cytoplasm of such cells is usually dark. Hexon antigen is arranged, 40 h after infection, in accordance with the central nuclear corpuscles and in the form of bright conglomerates in the wide region between the nuclear envelope and inclusion.

Projection and penton antigens are demonstrable 15-16 and 16-17 h, respectively, after infection; the nature of their distribution in the cell is essentially similar to the one described above for the hexon antigen [24-26]

The same pattern is observed in the quantitative study, which we were the first to conduct, of accumulation of different proteins, and a reliable increase in mean intensity of fluorescence is demonstrable by cytofluorometry

2-4 h after their initial appearance: examination of anti-hexon-serum-treated preparations revealed a reliable increase in mean intensity of fluorescence, to  $0.8 \pm 0.14$ , 16 h after infection (versus  $0.6 \pm 0.04$  in the control,  $p < 5\%$ ). A sharp increase in fluorescence, to  $3.8 \pm 0.3$  and  $5.0 \pm 0.3$  ( $p < 1\%$ ) is observed 17 and 22 h after infection. The mean intensity of fluorescence of penton base and fiber antigens increases appreciably 16-17 and 19 h after infection ( $1.8 \pm 0.1$  and  $3.1 \pm 0.15$ ,  $p < 1\%$ ). These indices also reach a maximum 22 h after infection [25, 27].

Consequently, the nature of distribution in the cell of the different capsid proteins is the same, but quite different from that of early protein. Capsid proteins appear in the cytoplasm of infected cells, then migrate to the nucleus. It should be indicated that the data we obtained were the first immunomorphological confirmation of the results of biochemical studies on translation of virus-specific mRNA in the cytoplasm and formation in it of polypeptides that are precursors of viral proteins, which then migrate to the nucleus [28-30], i.e., expression of the general patterns of protein synthesis in the cell in a model of a nuclear virus, such as adenovirus.

Determination has been made of some of the distinctions of formation of virus-specific mRNA coding synthesis of penton base and fiber and hexon of type 1 AB. For this purpose, studies were made of the effect of actinomycin D on synthesis of these proteins after brief addition of the antibiotic for one reproductive cycle (60-min treatment of infected cells with a solution containing  $1 \mu\text{g}/\text{ml}$  actinomycin D). It was shown that addition of the antibiotic in the first 8 h after infection totally inhibits transcription of the adenoviral genome. Addition of actinomycin D 9-10 h after infection already has a partial inhibitory effect, since the projection and hexon are formed in 7.6% and 10.0% of the cells, respectively.

On the basis of the obtained data and known conceptions on the mechanism of effect of actinomycin D on macromolecular synthesis, the hypothesis was expounded that formation of mRNA specific to the projection and hexon of type 1 AB begins between the 8th and 9th postinfection hours [31].

Investigation of internal proteins of DNA-genome viruses, i.e., basic proteins localized within the capsid, is important because of their close structural and functional link with virion DNA, on the basis of which their important role in virion morphopoiesis, stabilization of viral DNP and regulation of transcription of the viral genome is postulated [32, 33]. For this reason, we have been directing our efforts toward studying the internal protein of adenoviruses for the last 2 years.

A study was made of the effects of some chemical and physical factors on the structure of the capsid of type 1 adenovirus, in particular the process of degradation of type 1 AB in an alkaline medium. It was shown that there is destruction of some virions already after 10-min incubation of concentrated viral suspension at a pH of 10.0. At pH of 10.5-11.0, there is an increase in number of destroyed virions; the internal organization of the nucleoid

can be observed in some of them, and there are extensive accumulations of hexon per field of vision. With increase in pH to 11.5-11.8, this process is more intensive, and at pH 12.0 there is total disintegration of virions into separate capsomers. After 30-min incubation with 1.0% sarcosyl, there is degradation of adenovirion, which is characterized by swelling of the capsid, formation of "fissures" between it and the nucleoid and, finally, appearance of DNP [deoxynucleoprotein] with disintegrated structure. We also investigated the distinctions of the process of virion degradation after heating for 5 min at 56° and treatment with acetone (N. Ya. Spivak et al.). Such experiments are the first stage of obtaining internal protein in large quantities, since they are directed toward developing procedures for disintegration of viral particles. The study of the nature and dynamics of the degradation process will aid in gaining deeper understanding of the microanatomy of the adenovirion and its structural elements.

The main obstacle to such work is the difficulty of accumulating large quantities of purified virus, as well as development of methods of isolating and identifying internal protein. Methods have been refined for purifying virus in a cesium chloride gradient, by electrophoresis in polyacrylamide gel and chromatography on columns with KM-cellulose under elective conditions for isolation of basic proteins (N. P. Vantsak, G. G. Popenko, E. G. Mikhaylova). Chromatography on columns with KM-cellulose 52 of products of alkaline dissociation of partially purified type 1 AB yielded low molecular protein with a sedimentation coefficient of 1.1 S, which is similar, in some properties, to one of the components of adenoviral internal protein.

In the last 2 years, considerable attention was given as well to the study of the effects of DNA-genomal viruses and their components on the immuno-competent system.

The immunosuppressive effect of type 1 AB was demonstrated, which was tested on the basis of a decline of several indices characterizing the functions of the cellular and humoral systems of immunity in mice. Thus, administration of type 1 AB 5 and 8 days prior to administration of test antigen (sheep erythrocytes) elicited a statistically reliable attenuation of the reaction of hypersensitivity of the delayed type (DTH) as determined by the method of Lagrange et al. (1974), but addition of type 1 AB 2 days before the sheep erythrocytes did not affect this index. With inoculation of AB 5 days prior to sheep erythrocytes, there was also a statistically reliable decrease in number of cells producing hemolysins to autologous and sheep erythrocytes (AFC [antigen-forming cells]), as well as rosette-forming cells (RFC). The hemagglutinin titers were about the same in the experiment and control. The obtained data are indicative of the immunosuppressive action of adenoviruses, which may be considered one of the factors involved in their prolonged persistence in the macroorganism [34].

Another DNA-genomal virus, phage 62 of *Erwinia carotovora*, which we studied, also inhibits significantly reactions characterizing the state of cellular and humoral immunity, and phage proteins (phage "shadow" preparations) also have the same effect. Conversely, phage 62 DNA stimulates cellular immunity.

Thus, 10-day administration of 50  $\mu\text{g}$  DNA causes the indices of the DTH reaction to rise to  $0.78 \pm 0.095$ , versus  $0.48 \pm 0.029$  in control animals ( $p < 0.01\%$ ). These data are indicative of the difference in nature of influence of DNA-genome viruses and components thereof on the immunocompetent system (V. G. Dikovenko, N. Ya. Spivak and N. S. Dyachenko).

For the first time, it was shown that the DNA of phage 72 of Erw. carotovora induces interferon synthesis in vitro, in a culture of lymphocytes of the human palatine tonsils, and in vivo, in mongrel and inbred white mice. The maximum titers of interferon against 100 TCD<sub>50</sub> vesicular stomatitis virus are demonstrable 24 h after administration of DNA [36]. These studies were conducted in collaboration with Ya. L. Povolotskiy, doctor of medical sciences, and L. L. Krivokhatskaya from the Institute of Otolaryngology, Ukrainian Ministry of Health.

Phage 62 DNA has a marked inhibiting effect on development of transferable tumors, in particular Ehrlich's solid carcinoma [36, 37], as indicated by the statistically reliable decrease in tumor weight in the group of C57Bl mice given 50  $\mu\text{g}$  DNA daily for 10 days after transplantation of the tumor, as compared to tumor-bearing control animals ( $74 \pm 8.0$  mg and  $199 \pm 29.5$  mg,  $p < 0.01$ ).

The DNA studied transfects transferable human HeLa cells. This effect disappears after the preparation is incubated with DNAase. The DNA is a linear, double-helical molecule; according to electron microscopy data, its molecular weight is  $52.6 \cdot 10^6$  dalton; the tested DNA preparations had spectrophotometric indices of  $E_{260/280} = 1.8-1.82$  [35].

These data are indicative of the diverse biological effects of DNA of Erw. carotovora phage 62. Determination of the mechanism of this action will be the subject of our future investigations.

Thus, according to analysis of the submitted experimental data from the comprehensive study of structural components of adenoviruses and other DNA-containing viruses, the department of molecular biology of viruses has obtained the following principal results:

1. A correlation was demonstrated between the size of the projection [penton fiber] molecule of adenoviruses types 1 and 10 and the complexity of their antigenic structure, since a previously unknown sensitizing antigen was identified in the larger projection of AB type 1.
2. It was found that the type- and group-specific determinants of the hexon are oriented differently in the adenovirion, intracellular inclusions and monomeric capsomers.
3. Studies have been made of intracellular localization and dynamics of accumulation of different capsid proteins. Of basic importance is the demonstrated similarity of distribution of the latter in the cell and appearance in cytoplasm at the early stages of viral reproduction.

4. New data have been obtained on the different nature of effects of DNA-genome viruses (adenoviruses, certain phages) and components thereof on immune systems.

Analysis of the work done by the department for many years warrants the belief that, at the present time, it is one of the famous centers in our country for the study of adenoviruses. Work dealing with adenoviruses is being done in several institutes of our country (Institute of Virology, USSR Academy of Medical Sciences; Institute of Molecular Biology, USSR Academy of Sciences; Influenza Institute, USSR Ministry of Health) and in laboratories in Sweden, England, the United States, Hungary and GDR.; but our research differs in its complex approach and the of methods that permit the study of both adenoviral components and processes occurring in an infected cell. This enables our group to experiment in original directions. This approach is also applied to the recently begun series of experiments in our department to study the effect of DNA-genome viruses and their components on the immune system, a direction that is on the borderline between virology and immunology.

We believe that research in the department of molecular biology of viruses will develop in the following directions in the next 5-15 years: 1) structural and functional investigation of viral components; 2) investigation of molecular mechanisms of expression of genetic information of DNA-containing viruses in the presence of productive, various forms of nonproductive infection and heterologous cell systems.

The range of problems referable to the first direction, which is more relevant to the topic of this article, will include further structural and functional investigation of structural proteins of viruses, including adenoviruses, concurrently with determination of their antigenic specificity, investigation of specific antibody-producing reactions and involvement of antibodies to different proteins in neutralization of viral infectiousness; determination of the mechanisms of the immunosuppressive effect of DNA-containing viruses. It is planned to study viral DNP, principles of organization thereof, physicochemical characteristics and to determine the function of DNP proteins (internal virion proteins).

There will be intensive development, in the next few years, of research directed to the study of manifestations and mechanisms of effects of viruses and their components on the immune system, in particular, the study of the stimulating effect of DNA on immunogenesis and interferonogenesis, as well as transfection activity of viral DNA in heterologous cell systems, since it touches upon a virtually unexplored area, and we can expect the discovery of new manifestations of biological activity of DNA.

The obtained results will deepen our conceptions of the function of different components of the virion and of the nature of viruses as a whole; they will enable us to discover new manifestations of the dialectical correlation between the structure of viral macromolecules and their specific functions.



Investigation of molecular properties of viral proteins has much applied significance, since it can aid in further progress in such important directions of practical virology as refinement of ways and means of diagnosing and providing specific prophylaxis of viral infections. It can be expected that the use of diagnosticums of purified individual viral proteins and specific sera to them will augment the specificity of serological and immunofluorescence analysis techniques, which are often used as well in diagnosing adenoviral infections, since there are no antibodies to contaminant nonviral proteins in such sera.

It should be noted that adenoviruses are rather widespread; they induce primarily lesions to the respiratory tract varying in symptoms and severity of clinical signs. Adenoviruses may be the cause of local epidemic outbreaks or sporadic cases of acute respiratory disease. Adenoviral infection often accompanies influenza and complicates its course in children, particularly younger ones. In children, adenoviral infection is not infrequently of the pneumonia type, and it can lead to death [2].

As we have already stated, monospecific sera to individual capsid proteins of adenoviruses were developed by us in our country [25, 27, 38]. One of these antisera, namely serum to the hexon of type 1 AB, was used for immunofluorescence demonstration of adenoviruses in infants admitted to the clinic of the Institute of Pediatrics, Obstetrics and Gynecology imeni Prof M. P. Buyko, Ukrainian Ministry of Health, with the diagnosis of acute respiratory disease or pneumonia. We conducted these studies in collaboration with members of the staff of that institute, N. T. Kucherova, candidate of medical sciences, and T. A. Motuz, doctor of medical sciences.

In all, 230 sick children were screened between October 1975 and March 1977. Adenoviral antigen was found in epithelial cells of the upper respiratory tract (columnar and occasionally squamous) in 57 children, which constitutes 28.4%; antigen was detected in 44% of the children surveyed during the fall and winter of 1976-1977, during an influenza epidemic. Concurrently, we treated the same smears of material from the throat with antiadenoviral FITTs [expansion unknown]-globulin obtained against unpurified extracts of infected cells, which contain whole adenovirions, capsid proteins and a broad spectrum of contaminant antigens of cell origin. Adenoviral antigen was demonstrated in 20 cases (8.7%,  $p < 0.1\%$ ). Consequently, the use of anti-hexon serum made it possible to augment significantly the productivity of immunofluorescent demonstration of adenoviruses in children [39].

Evidently, the use of monospecific sera is also promising for other serological reactions used in diagnostic practice, and for this reason extensive trial of such sera will be one of our tasks in the near future.

In-depth knowledge of molecular properties of viral proteins is important to refinement of preventive antiviral preparations. The method of preparing vaccines from purified surface proteins of viruses is promising, although rather complicated. Such vaccines have several basic advantages over live



(attenuated) or killed (inactivated) vaccines made of viral particles, since they do not contain viral nucleic acids, possible contaminant viruses and allergenic cell proteins. The lack of a complete viral genome renders them noninfectious and deprives them of potential oncogenicity and mutagenicity. In the last few years, data have been obtained on the depressive effect of viruses on the immune system, in particular, the functional activity of systems of cellular and humoral immunity. The successful experiments in gene engineering are indicative of the feasibility, in principle, of developing hybrid viral genomes, and this possibility should also not be overlooked in evaluating the future of vaccines made of viral particles. In addition, the real possibility of developing complex preparations of proteins from different viruses, which is particularly important in immunizing children [40], is another advantage of protein (subunits, chemical) vaccines.

At the present time, there has been theoretical substantiation of the possibility of developing vaccines from the penton fiber and hexon of adenoviruses [18, 41, 42]; their high immunogenicity and marked protective effect have been demonstrated. The use of such protein vaccines is particularly important in groups of children, in whom adenoviral infections are characterized by a high incidence and serious clinical course.

Vaccines made of proteins of other viruses--hemagglutinins of influenza virus [43-47], measles virus [48], subunits of rabies virus [49]--are also effective. We have discussed this question previously in great detail [40].

It can be expected that the patterns we established in our structural and functional study of capsid proteins of adenoviruses, namely the correlation between size of the protein molecule and complexity of its antigenic structure, changes in orientation of antigenic determinants of different capsid proteins depending on their spatial structure and details on the process of immunoinactivation of the virion, will also apply, to some extent, to other icosahedral viruses (polyomyelitis, herpes group viruses, rhinoviruses), as well as to viruses with helical type of symmetry (influenza virus and others).

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REPRODUCTIVE CAPACITY OF PUCCINIA GRAMINIS PERS. F. SP. TRITICI ERIKS, ET HENN. ON WHEAT CULTIVARS WHICH DISPLAY DIFFERENT RESISTANCE TO STEM RUST

Leningrad MIKOLOGIYA I FITOPATOLOGIYA in Russian Vol 12, No 2, 1978  
pp 132-136 manuscript received 26 May 1976

[Article by S. S. Sanin, A. S. Kaydash, V. P. Chuprina and F. A. Babina]

[Text] The capacity of rust diseases of grain plants for rapid spread over a substantial territory is due, to a considerable degree, to the high reproductive capacity of their causative agents.

Based on data of Stakman (1934), one uredopustule of *Puccinia graminis* f. sp. *tritici* Eriks. et Henn. fungus, alone, can form about 100 thousand uredospores. According to reports by Christensen (1942), each uredopustule of this species contains from 50 to 400 thousand spores and, according to testimony of E. Goyman (1954), in 24 days of sporogenesis in the uredopustule up to 200 thousand uredospores are formed.

The cited literature data reflect the sporulating capacity of the fungus on receptive cultivars of the plant host.

Reproductive capacity of phytopathogenic fungi is subject, however, to great fluctuations. It depends on stability of cultivar, degree of plant infection and various factors of the environment (Yarwood, 1961; Rapilly, et al., 1970).

Based on data of K. M. Stepanov (1962), these fluctuations, naturally, affect the dynamics of the corresponding diseases and recording of fungus sporogenesis in each concrete case is important for revelation of the causes and peculiarities of outbreaks of the diseases and for lowering them.

In our own research we have attempted to evaluate the sporulating capacity of *P. graminis* f. sp. *tritici* fungus on cultivars of wheat which vary in resistance to rust.

The method used to measure sporulating capacity of rust fungi has already been described in detail by us (Sanin, et al., 1975). It involves isolation

of the affected portion of the stem in a spinal chamber.

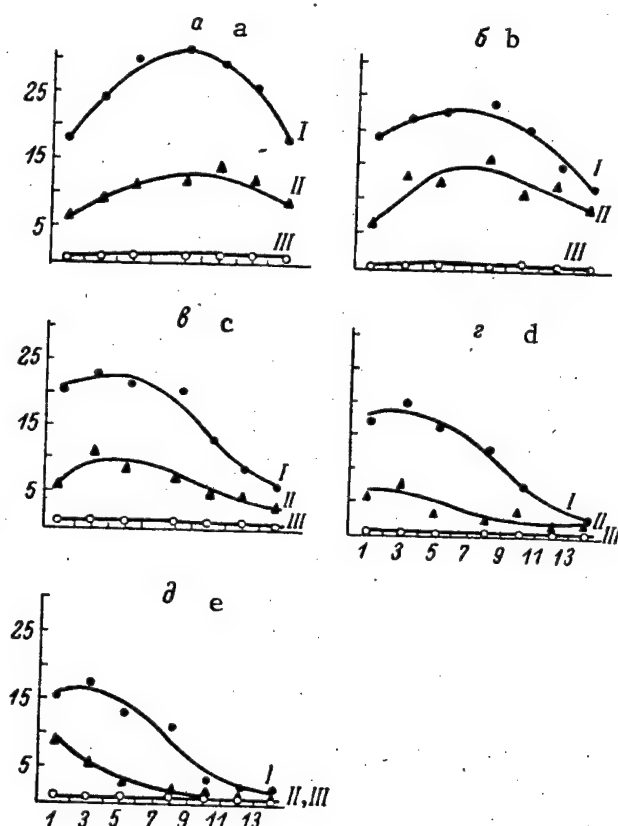


Fig. 1. Dynamics of formation of uredospores of *P. graminis* f. sp. *tritici* on cultivars of wheat with different resistance to stem rust

abscissa--periods of observation (in days); ordinate--formation of spores in one pustule (in thousands).

a--5%; b--25; c--50; d--75; e--100%. Cultivars: I--Kubanskaya-3 (type of infection 4); II--Markiz (type of infection 2-3); III--Vernal (type of infection 1).

Ditto for Figs. 2 and 3

The isolator chamber is a glass tube, diameter 20-25, length 200-250 mm. The tube is covered, on the outside, from the upper surface, with a thin layer of white oil paint. This covering provides dispersed light in the tube and protects the plants from burns.

The isolator containing the affected part of the stem is set in a horizontal position on wire supports. Everyday, the formed spores, without



removal from the tube, were shaken off with light tapping on the stem and then washed with 20 ml ethyl alcohol. The number of washed spores was counted under the microscope in a Goryayev chamber. Observations for spore formation on the damaged parts were carried out for 14 days. Simultaneously, count was taken of the number of powdering pustules.

The studies were carried out on three cultivars of spring wheat from a collection of basic cultivar-differentiators of stem rust: Kubanka-3, Markiz and Vernal. These cultivars displayed different sensitivity to race 39 used in the experiment. According to the Stakman and Levin scale (1944), they gave, with this race, types of infection 4, 2-3 and 1, respectively. Isolators were set up for plants which had varying degree of infection: 5, 25, 50, 75 and 100%. Estimate of degree of development of the disease with the various types of infection was done according to the Peterson, et al. scale (1948).

Based on data in Fig. 1, the higher the resistance of the plant host and, correspondingly, the lower the type of infection, the fewer the spores formed in each uredopustule during the period of spore formation. Thus, for example, during a 5% intensivity of disease development, the number of spores forming per day varied, in the Vernal cultivar (type of infection 1), from 0.5 to 1.5 thousand, in the Markiz cultivar (type of infection 2-3), from 6.0 to 12.0 and in the Kubanka-3 cultivar (type of infection 4), from 17.0 to 30.0 thousand.

With slight infection (5-25%), the curves which characterize the sporogenicity of the fungus in time in all the cultivars had almost a true parabola shape. The intensity of spore formation at first rose steadily and then, reaching a maximum on the 6-8 day, also gradually declined.

With severe damage (50-100%), formation of spores reached a maximum far more rapidly, even on the 2-3rd day after opening of the pustules, following which it rapidly decreased.

The lines which depict the dynamics of sporogenesis were positioned, in this case, substantially lower with respect to the abscissa axis. This indicated that, with increased affection, the number of uredospores, which form in one uredopustule, decreased.

With an increase in degree of plant infection, the duration of the period of spore formation decreased. Whereas, at 5% affection, a rather severe sporulation was noted even after 14 days, at 75-100% the period of spore formation dropped to 10-11 days.

Fig. 2 presents data which characterize the average number of spores forming in the sporulation period in one fungus pustule in 1 day.

On the resistant Vernal cultivar with type of infection 1, the fungus produced less spores--by a factor of 10-20--than on the average-susceptible

Markiz wheat (type of infection 2-3) and less--by a factor of 30-40--than on the susceptible Kubanka-3 cultivar (type of infection 4). Whereas, in type of infection 4, there were formed in one pustule per 1 day--depending on the degree of plant affection--from 10.0 to 24.0 thousand spores, in type 2-3 there were formed from 3.0 to 12.0 thousand, and in type 1, only from 0.1 to 2.0 thousand spores.

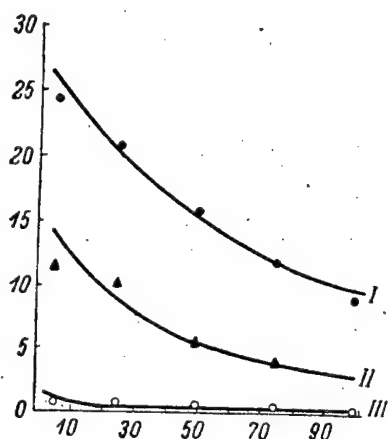


Fig. 2. Average number of uredospores of *P. graminis* f.sp. *tritici* forming in the period of sporulation in one pustule under different degree of plant affection. abscissa--degree of plant damage (in %); ordinate--number of spores forming (in thousands)

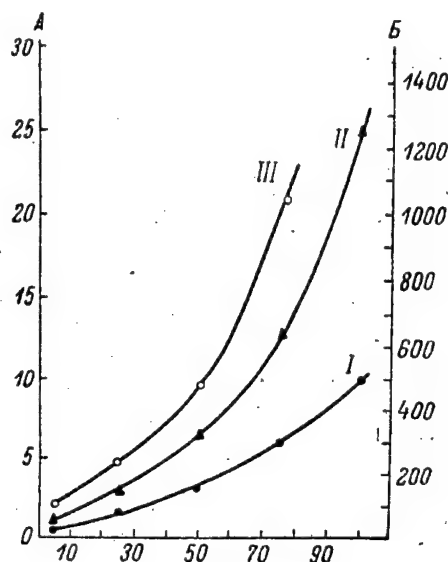


Fig. 3. Number of uredopustules of the causative agent of stem rust which form on the diseased stem of wheat under different degree of plant affection. abscissa--degree of affection (in %); ordinate--: A, amount of pustules on 1 cm of stem surface (in numbers); B, amount of pustules per one stem (in numbers per 50 cm)

With an increase in plant infection, the intensity of spore formation by each uredopustule decreased. Whereas, as an example, with the degree of infection equal to 5% an average of 24.0 thousand spores in one pustule formed on Kubanka-3, with a 50% degree, 16.0 thousand formed, and at 100% only 10.0 thousand.

Fig. 3 shows that there is not a direct linear relationship between number of pustules per unit area of stem and degree of its infection. With an increase of infection the amount of uredopustules decreases. For this reason, with an increased degree of infection, by a factor of 2 as an example, the number of pustules increases more than double.

The higher the cultivar resistance, the lower the type of infection, and the less the quantity of uredopustules, the greater did their number guarantee one or another infestivity. For example, at 75% infestivity, on one stem of Vernal cultivar (type of infection 1), with a height of 50 cm, there were formed more than 1000 uredopustules; on Markiz wheat (type of infection 2-3) only 700 pustules, and on Kubanka-3 cultivar (type of infection 4) only 300 pustules.

Despite the fact that the substantially larger number of pustules on resistant cultivars per unit of stem area corresponds to an equal degree of infection than on susceptible cultivars, the number of fungus spores formed in the first case is considerably lower than in the second case. Thus, for example, in type of infection 1, fewer spores--by a factor of 4.0-10.3--were formed per one affected stem than with type of infection 2-3; it was less--by a factor of 5.0-12.4--than with type of infection 4 (see Table).

Average number of uredospores formed by the fungus *P. graminis* f. sp. *tritici* in the course of 1 day on cultivars of wheat which differ in resistance to stem rust.

1. Степень поражения растений (в %)	2. Количество спор (в тыс. штук)					
	3. Кубанка-3 (тип инфекции 4)		4. Маркиз (тип инфекции 2-3)		5. Вернал (тип инфекции 1)	
	а. в одной уредопустуле	б. на одном стебле высотой 50 см	а. в одной уредопустуле	б. на одном стебле высотой 50 см	а. в одной уредопустуле	б. на одном стебле высотой 50 см
5	24.0	600	12.0	480	1.0	120
25	21.0	1680	10.0	1400	0.6	135
50	16.0	2720	6.0	1860	0.5	245
75	13.0	3900	4.0	2480	0.3	390
100	10.0	5000	3.0	3750	0.1	—

- Key
1. Degree of plant affection (in %)
  2. Number of spores (in thousands)
  3. Kubanka-3 (type of infection 4)
    - a. in one uredopustule
    - b. on one stem, 50 cm high
  4. Markiz (type of infection 2-3)
  5. Vernal (type of infection 1)

The data shown indicate that the cultivar peculiarities of the plant host have great importance in the dynamics of formation of spores of the causative agent of stem rust on infected plantings of wheat. On resistant cultivars manifesting a lesser type of infection to disease, the fertility of the phytopathogenic fungus decreased substantially.

This circumstance serves, evidently, as a very important factor restraining

the appearance of pest- carrying epiphytes of this disease on resistant wheat cultivars.

#### CONCLUSIONS

The reproductive capacity of the fungus *P. graminis* f. sp. *tritici* depends on the resistance of the plant host. The higher the resistance and, correspondingly, the lower the type of infection, the fewer number of spores are produced by the pathogen.

With type of infection 4, the causative agent of stem rust of wheat forms from 10.0 to 24.0 thousand spores per one uredopustule of the fungus per day. With types of infection 2-3 the daily productivity of pustules is from 3.0 to 12.0 thousand spores, and with type 1 it is only from 0.1 to 1.0 thousand. On one affected stem of a resistant cultivar there are formed fewer spores--by a factor of 4.0-10.3--than on a stem of average susceptibility, and fewer spores--by a factor of 5.0-12.4--than on a susceptible cultivar.

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SURVEY OF THE RACES AND VIRULENCE GENES OF RUST AND POWDERY MILDEW ON WHEAT

Leningrad MIKOLOGIYA I FITOPATOLOGIYA in Russian Vol 12, No 2, 1978, p 182,  
manuscript received 6 Jan 78

[Article by N. N. Guseva, All-Union Institute of Plant Protection, Leningrad]

[Text] A conference of representatives of the CEMA countries on the problem "Development of Principles of Resistance of Plants to Diseases" took place 13 to 16 December 1977 at the All-Union Scientific Research Institute of Plant Protection (Leningrad-Pushkin).

Participating in the activity of the conference were leading immunologists who work on the survey of the races of rust and powdery mildew fungi on wheat. These included Doctors K'rzhin (BPR), Bocha (HPR), Meyer (GDR), Kvyatkovski (PPR), Sebesta (CSSR) and others. The USSR was represented by academician M. S. Dunin, by directors of immunity laboratories L. T. Babayants, V. I. Krivchenko, M. P. Lesovoy and A. A. Voronkova. Slovenchikova (CSSR) and Falkenstein (GDR) presented their suggestions on survey of races of yellow rust and powdery mildews.

In planning of selection programs directed toward the creation of cultivars resistant to the diseases, serious difficulties include the processes, constantly arising in fungal populations, of appearance of new races, biotopes and genes of virulence. Genes of resistance of international cultivars-differentiators of rust and powdery mildew fungi are practically not used in selection, either by us in our country or by countries of Central Europe. Hence they have not shown a directed influence on populations of fungi. For the majority of selection programs, directed to control the diseases cited, it is not possible to use a series of almost isogenous lines created on the American continent. Reports on the creation of genetic differentiators are the only ones published in our country.

In 1975, at the preceding conference in Maysdorf, resolutions were adopted on organization of operative information selection centers on changes occurring in populations of fungi, with the help of cultivars-separators. Apart from effective collection of cultivars, unification is important of the changes arising in populations of fungi. Insofar as recording is possible of the infectious origin from adjacent countries immunologists should speak, in identification of pathogens, "in one language". The following order of the survey of races has been accepted for a two-year period.

1. Powdery mildews (cultivars-differentiators):

Bartweizen-Salzmunde 14/44 (mir), Ulka (Mlu-Pm2), Axminster (Mtl-Pm 1), Halle st. 13 471 (mlha + Mid), Weißen-stephan M.-Tr. cartholicum (Mle-Pm4), Hope (mlk-Pm5), Chul (Mle-Pm 3c).

2. Brown rust of wheat (cultivars and lines):

Mediterranean (CI 3332), Democrat (CI 3384), Hussar (CI 4843), Malakof (CI 4898), Carina (CI 3756), Brevit (CI 3778), Laros (CI 3779), Webster (CI 3780), Lr 1, Lr 2<sup>4</sup>, Lr 3D, Lr 9, Lr 10, Lr 14b, Lr 16, Lr 19, Lr 23, Lr 24, Kavkaz and Khar'kovskaya 46.

3. Stem rust (lines and supplementary cultivars):

Sr 5, Sr 6, Sr 9b, Sr 11, Sr 13, Sr Ft I, Sr SB (Salzmuender Bartweizen), Srd IV (Khapli), Arthur 71 CI 15 282, Purdue Abe, Era and Bezostaya 1.

4. Yellow rust (cultivars-differentiators):

Salzmuender 14/44 Chinese 166, Bon Fermier, Hybrid 46, Tadorna, Ibis, Flevina, Michigan Amber, Moro, Vilmorin 23, Carsten V, Triticum spelta album, Sunwon x Omar, Zely, Sinnetou-4, Heines Peko, Lee and Compair.

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## PSYCHIATRY

### BOOK REFUTES 'SLANDEROUS FABRICATIONS' ABOUT FORCED COMMITMENT

Moscow SOVETSKAYA YUSTITSIYA in Russian No 8, 1978 signed to press 5 Apr 78 pp 30-31

[Article by Ye. Kholodkovskaya, professor and doctor of medicine, and P. Pashkevich, candidate of law and distinguished RSFSR lawyer, in the column "New Books": "B. A. Protchenko. 'Compulsory Medical Measures' ("Yuridicheskaya literatura", 1976, 104 pp)]

[Text] Since the issues of employing compulsory medical measures are among the most difficult, the library of the people's judge has been enriched by a necessary and beneficial book for practicing officials of agencies which uphold the law.

The textbook under review thoroughly examines the grounds in criminal law and the procedural rules for the court to employ (impose), extend, change and countermand compulsory medical measures with respect to persons who have committed socially dangerous acts as specified under criminal law, but who have been found to be of unsound mind, and also, to persons who have become mentally ill after committing a crime, inasmuch as these persons, in contrast to those who are of unsound mind, are guilty and can have criminal proceedings brought against them after their recovery. Moreover, several authors who were confused on this issue are correctly criticized.

The book examines the types of compulsory medical measures in detail; it points out that they are only employed in the event it is established--before the court reaches its verdict--that the person who committed the socially dangerous act, which is deemed to be a crime by law, is mentally deranged. While resolving the issue of the need to employ said measures against a person who has been found to be of unsound mind, the court proceeds from the fact of whether the person's mental condition represents a danger to the people around him. The nature and gravity of the act do not play a role in this. A

court determination that the person's mental condition has ceased to be dangerous to society rules out the possibility of employing compulsory medical measures against him. Concerning a person who has become mentally ill after committing a crime, the need to employ compulsory medical measures against him is determined by considering his mental condition and also the nature and gravity of the act.

The author also points out that no matter what danger a mentally ill person presents to those around him, based on the extent of his illness, compulsory medical measures cannot be employed against him if he has not committed a socially dangerous act as specified by law or if it has not been proven that he committed such an act.

The work also thoroughly elucidates the legal proceedings for cases of the given category during all their stages and it provides methodological recommendations. At the same time, a great deal of attention is devoted to thoroughly preparing for trial of a case on employing compulsory medical measures. These preparations must prevent the trial of unsubstantiated cases in a court session and they must rule out the possibility of violating the rights of the mentally ill.

The issues relating to the trial of this category of cases in courts of primary and appellate jurisdiction are also examined.

The book convincingly demonstrates that, under Soviet criminal law, forced commitment of a person who has committed a socially dangerous act--as specified by the Criminal Code--to a psychiatric hospital for treatment is only permitted based on a court ruling which is delivered during a session of the court on the basis of a complete, comprehensive and objective investigation of the case material, while observing the general and special rules for legal proceedings which create a real guarantee for observing the rights and legal interests of the mentally ill.

Compulsory medical measures are not a penalty, a punishment for the act; they are employed to restore the person's disturbed health and to prevent the repetition of acts which are dangerous to those around him. Besides the courts, no other agency in the USSR is endowed with the right to commit people to psychiatric hospitals for the employment of forced measures of treatment.

All of this decisively refutes the slanderous fabrications of our enemies that cases of committing mentally healthy people,



the so-called dissidents, to psychiatric hospitals is supposedly permitted in the Soviet Union.

While dwelling on the issue of appellate review of the probity of a verdict of a court of primary jurisdiction, the author points out that the rule on the proscription of a turn for the worse (art 14 of the Principles for Criminal Proceedings which forbids appellate courts from increasing the punishment as well as from employing a law on a graver crime) does not affect cases of the given category since there is no charge and punishment is not levied in cases on employing compulsory medical measures. Moreover, such measures are employed in the interests of more effective treatment of the patient. Therefore, if it is established during appellate review of a case that, contrary to the case material which attests to a given person's particular danger to society, the court of primary jurisdiction mistakenly sent him to a general-purpose psychiatric hospital instead of a special-purpose psychiatric hospital, then the appellate court can change the type of psychiatric hospital itself. In this case, countermanding the incorrect verdict of the court of primary jurisdiction and returning the case to it for a new trial would only entail an unnecessary complication of procedural processes and, for all practical purposes, an unnecessary retrial of a case which has already been investigated in sufficient measure in a court session.

One of the textbook's chapters is devoted to the court's employment of forced treatment for alcoholics and drug addicts who have committed a crime while of sound mind. It points out that the employment of this measure, along with punitive measures for the crime committed by the guilty party, significantly increases the preventive effects of sentences and it serves as an important weapon in the struggle against alcoholism and drug addiction.

The textbook also examines the issues of the court's imposition of forced treatment in work-treatment dispensaries for chronic alcoholics and drug addicts who have not committed a criminal offense but are evading mandatory attendance for special treatment and are violating labor discipline, public order and the rules of socialist society in spite of the disciplinary, social or administrative actions taken against them.

While pointing out the merits of the book under review, we also believe that specific propositions contained in it are debatable. Thus, in our opinion, the author's arguments on the employment of an act of amnesty in respect to the mentally ill are incorrect. He is correct when he writes that an act

of amnesty does not affect a person of unsound mind who is undergoing forced treatment in connection with a socially dangerous act committed by him which falls under the amnesty. And he is completely justified when he maintains that "in contrast to prisoners, persons who are being subjected to compulsory medical measures cannot be amnestied" (p 30). But, the author's argument on how one should act on those cases where the case has not been finally decided, but is in the trial stage, cannot be linked to this proposition. In all such cases, in his opinion, if an act of amnesty eliminates the application of punishment for the perpetrated act, it also rules out the possibility of employing compulsory medical measures against the person who committed said socially dangerous act while of unsound mind (see pp 28-30). Therefore, he believes that all such cases should be stopped during the preliminary investigative stage and, if the case has been brought to trial, then it should be stopped in the court (p 29). "From the humane point of view," writes B. Protchenko, "it is impermissible that amnesty only alleviates the situation of those persons who committed a crime while of sound mind, and it has no effect on persons who committed a socially dangerous act in a state of illness which made it impossible for them to be aware of their actions or to control them." (p 29).

This position of the author of the textbook cannot be regarded as consistent and convincing. We believe that those people who proceed on the basis that an act of amnesty--which eliminates the application of punishment for guilty parties--does not rule out the need for imposing and employing treatment under forced conditions for persons who have committed a socially dangerous act in an unsound state of mind are more correct in their resolution of this issue. And there is nothing inhumane about this since treatment is not punishment.

However, it should be pointed out that the supporters of this point of view are forgetting the requirements of part 3, art 410 of the RSFSR CCP /Code of Criminal Procedure/ which does not correspond to their position. Evidently, the given issue requires a more precise legal solution.

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## DISEASE AS A FORM OF A SYSTEM

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[Article by I. G. Beshpal'ko]

[Text] A number of complex problems which cannot be solved by the traditional, predominantly analytical methods have arisen in the last few years in various areas of science and practical activity. The systems approach principle, which was initially oriented on integrated study of phenomena in relation to the entire diversity of their relationships and which opened the door to theoretical synthesis of empirical data, attracts special attention in this connection. Although some of the fundamental conceptions of the systems approach had arisen as long ago as in the 1920's-1930's (a significant contribution was made to the systems approach by P. K. Anokhin, 1935; Bertalanfi, 1937), the general scientific trends which aroused the high interest in this direction did not mature until now. Development of a number of modern areas of knowledge (cybernetics, information theory, theoretical biology, and so on) prepared scientific thought for perception of new fundamental ideas such as "control," "information," and "systems," which go beyond the particular sciences. It is becoming increasingly more obvious in this case that, for example, at all levels beginning with the subcellular, the world of biological phenomena is profoundly systemic. Thus the general theoretical principle of systemic organization of natural and social phenomena was created and strengthened; because of its generality, it approaches philosophical generalizations. The systems approach is interpreted by Soviet authors as a natural methodological confirmation of the principles of dialectical materialism, among which the notion that phenomena in nature and society are deeply interrelated occupies a central place.

Naturally, the basic concept of the systems approach is the "system," which has a high degree of abstraction and generality, and which pertains to objects and phenomena of all natures. A system is some integral whole consisting of interacting parts (having in mind interactions involving energy and information). The properties of a system are not reducible to the properties of parts artificially isolated from it (elements, subsystems); instead, the properties of the elements of the system and their relationship are determined to a significant extent by the system as a whole and by laws inherent to it as a whole.

The methodological significance of the principle of systemic organization lies not only in that it affords a possibility for systemic integration of empirical data at an objective theoretical level (in contrast to the statistical methods of factor and regression analysis, which produce formal integration), but also (this is especially important today) in that the phenomena being studied are generally beginning to be thought of as systemically organized, which makes them appear in a new, sometimes unexpected light.

Application of the systems principle to medicine in general and to psychiatry in particular boils down today to two basic aspects: 1) The systems approach in organization of medical care (therapeutic, rehabilitational, preventive); 2) systems analysis of the protective features of the body and the personality in the fight against disease. These two approaches are sometimes closely intertwined, for example in organization of rehabilitational measures.

There is, however, another theoretically and practically very important aspect of the problem--interpretation of disease itself as a type of system.\* When we look at disease as a system, we immediately face a number of fundamentally important problems. First of all the main property of any system is that it is stable and it is capable of adaptation through experience. Thus when we view disease as a system, we presuppose that disease is capable of active, to one degree or another, adaptation to the body's changing environment--its protective reactions, therapeutic influences upon it, and so on. Hence interpretation of disease as a system requires development of new therapeutic strategies accounting for the active adaptive capabilities of the pathological (and nosological) process.

The fact that disease is a type of functionally organized system ("functional" in the sense that, similarly with functional systems as defined by P. K. Anokhin, it makes use of some of the body's particular physiological mechanisms in its temporal organization) is confirmed by a number of arguments, which would be suitable to cite here. Disease, as defined by I. R. Petrov, is the unity of destructive and adaptive phenomena (coupled with an insufficiency of the latter). The definition of disease presupposes two factors--destructive and protective or "defensive" (as defined by I. P. Pavlov). The pathogenesis of disease is characterized by development of stereotypic reactions that are not a direct and indirect response to harmful influences but which have their own laws, are capable of self-movement and self-development and, once they arise, develop in accordance with the law of chain reaction. This provides the grounds for assuming that pathogenic mechanisms develop in accordance with certain programs implemented owing to the mechanisms of self-regulation. This programmed and stereotypic nature of pathogenic

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\*Disease is interpreted in this context as a pathological (and nosological) process; the general definition of disease as a unity of destructive and adaptive phenomena is not implied. These issues are discussed below.

mechanisms indicates that they had evolved through evolution, and that they reflect many centuries of creation of the body's adaptive reactions, which to a certain extent pathological and nosological processes are (I. V. Davydovskiy).

A number of premises should be discussed in connection with my objective of specifying the possible role, within a number of today's concepts, of the idea that a pathological process is an independent functional system. Secondly we should emphasize that according to Davydovskiy, pathogenic processes (identical to a certain extent with pathological and nosological processes) proceed owing to the mechanisms of self-regulation--that is, according to today's ideas they are organized as systems. This agrees with the ideas presented here, but there is a major difference, consisting of the following. Davydovskiy's assertion that pathological and nosological processes are adaptive reactions of the body elicits a number of serious arguments. This can be seen first of all in clinical experience.

The logic followed in defining pathological reactions as adaptive reactions is based on general biological ideas according to which all evolutionarily confirmed reactions of the body must be adaptive: Were the case otherwise, they would have been eliminated by natural selection. Thus a contradiction arises between clinical experience, clearly arguing against the adaptive significance of pathological processing, and the ideas of general biology. This can also be viewed as a contradiction within clinical experience based on the ideas of general biology: On one hand there is the clinical evidence that pathological processes are evolutionarily confirmed and, being so, they are adaptive as well; on the other hand they are doubtlessly harmful to the body.

The solution to this contradiction is as follows. True, all of the grounds exist for assuming pathological processes to be evolutionarily confirmed and adaptive, but it is not in relation to the body that they are adaptive. It could be thought that pathological systems are not systems of the body in the full meaning of this word, but that instead they are relatively independent systems existing in the body on the basis of its functional mechanisms and having their own evolutionarily confirmed laws of development. From this point of view the picture of disease as a whole, which embodies the unity of destructive and adaptive phenomena, is the sum of the interaction (struggle, adaptation) of the body's protective powers and a pathological functional system parasitizing the body. Modern empirical data confirming these ideas are presented below.

I have dwelled on the marginal but fundamentally especially important notion of a pathological process as a system foreign to the body, one that just capitalizes on its functional mechanisms. Moreover a second, "intermediate" idea, according to which certain systems of the body having doubtless adaptive significance to the body fall out of the control of the integrated whole in the presence of disease and, acquiring autonomy, become disadaptational in relation to the body is very probable. The contradictory combination of the characteristics of their evolutionary confirmation and their disadaptational role becomes understandable in such cases. The subsequent discussion will pertain basically to the first "marginal" idea.

Thus, let us begin with the idea, one which is natural and which does not generally elicit doubt in medical practice, that disease is disadaptational in relation to the body. We assert in this connection that disease, viewed as a type of pathological functional system, develops and improves its mechanisms in the course of evolution and possesses its own capability for adaptation. We would have to delineate two aspects of systemic evolution and adaptation in this connection--adaptation to the external influences experienced by the system as such, which is mandatory for any system by definition, and adaptation of a supersystem, with which the system under examination may be associated one way or another. In a general case these two types of systemic adaptation do not necessarily coincide. On the whole, in the process of evolution, formation of systems which are associated with a supersystem and which make use of its mechanisms but which have only the goal, mandatory to any system, of that system's own adaptation ("parasitic systems") is fully permissible. When such a system is disadaptive in relation to the host, we refer to this phenomenon as disease. Within the limits specific to such a system we find only an information "nucleus" defining the "goal" of the system--that is, mainly its adaptation within the body, and the concrete way in which the structures of the host are to be used. Data exist confirming the possibility of such systems. We should recall in this connection the modern ideas concerning the extremely great nonspecificity of genes, and the idea that, in connection with this, introduction of foreign genes into the host's chromosomes coupled with introduction of foreign information is possible. This is the crux of the debate on gene engineering. These ideas are based in particular on "wandering" genes, for example of viruses capable of including themselves within the chromosomes of the host. In V. L. Ryzhkov's opinion the doubtless authenticity of these facts does not make them more understandable. It can be thought, however, that one of the important mechanisms of disease pathogenesis, namely creation of the information nucleus of a system intimately including itself within the dynamics of the host, is based on introduction of foreign information. Experimental laboratory data confirming these ideas have arisen quite recently. Their essence lies in the following. Viruses are unique in that they all experience a stage in which genetic material is the sole binding link between generations. In order to produce viral progeny, a viral genome penetrating into a cell alters the anabolism of the host cell for synthesis and assembly of viral components. Thus the host cell becomes in part a system foreign to the body, and it performs functions foreign and harmful to the host. In other words a pathological functional system, as defined above, develops.

The idea that disease is a functional system permits us to discuss causality in medicine, which is an extremely acute problem in modern psychology, more broadly. It would be sufficient to recall in this connection the debate on Davydovskiy's article "The Problem of Causality in Medicine (Etiology)." In the most general form, three types of causal relationships are distinguished in modern scientific philosophical knowledge: 1) The strict mechanical causality of classical mechanics; 2) the probabilistic causality of quantum and subquantum phenomena; 3) "quasipurposeful" or cyclic causality within the framework of organic determinism, which is the conception of determinism in dialectical materialism applied to biology (I. T. Frolov).

The first, mechanistic type of cause-and-effect relationships is based on the idea that energy is transmitted and that movement corresponding to the type of energy transmitted (electrical, mechanical, and so on) is elicited in matter. Successive hits of billiard balls placed in a row or a chain of chemical reactions is a natural model of such cause-and-effect relationships. Notions of causality of this type dominate in medicine today. What this implies is that disease is elicited by a causal factor acting in the form of some initial impulse, or constantly (for example microflora in an infection process). Development of disease is interpreted as various cause-and-effect series, chain reactions, avalanche processes, and so on. The idea that disease is a "blind" process, the direction of which depends only on the specific features of the interacting components, is common to all of these ideas. Thus the task of fighting disease naturally boils down to eliminating the initial or intermediate causes and breaking the pathological cause-and-effect chain.

Naturally, it is not presupposed in this case that disease, viewed as a systemically organized phenomenon, is capable of a certain amount of adaptation, of opposing adaptation of the body viewed as an integrated, systemically singular phenomenon, because this idea goes beyond this mechanistic type of cause-and-effect model. I. V. Davydovskiy argues against these mechanistic ideas from a general biological point of view; his own ideas, however, were also incomplete from the standpoint of clinicists.

I will not dwell on the second, probabilistic type of cause-and-effect relationships, which are a topic of inconclusive debates in physics and philosophy. To a certain extent the third type of cause-and-effect relationships includes within itself the first two types of causality, but the latter are significantly supplemented by a new, specifically biological content. Not only the "prodding" force typical of the first type of causality but also "controlling" information (negentropy) is mandatorily present in this last type of cause-and-effect relationship. These two components (energy and information) are of equal value. This informational type of causality is closely associated with one of the most fundamental properties of living matter--mandatory presence of two levels of the organization of matter--the level of matter and energy and the level of information--in all biological objects. "When we enter the world of biological objects, the simple 'single-level' atomic matter of physics and chemistry becomes significantly enriched, acquiring a more elaborate, differentiated form: Thus we find that structures of the organic world are associated with at least two levels of atomic organization correlated with one another. One of these is the 'material' level...of proteins existing as the real material carriers of the phenomena of life we know today. The other level, meanwhile, is that of information potential, a more delicate level basically containing the encoded discrete description of all 'future' states of this 'material' protein level" (A. I. Akchurin). Thus the specific features of the structure of living matter themselves predetermine the type of cause-and-effect relationships existing in biological objects.



The hypothesis that disease is a functional system agrees mainly with this type of biological causality. In connection with the above, the central issue of pathogenesis is that of information that creates and maintains the pathological process. I had noted earlier that we can imagine that there is such an extreme type of functional system, of which only an information nucleus is specific, with all other mechanisms supported through the use of the host's mechanisms. I had presented an example of such a genetic information "nucleus"--the viral genome. Other types of information besides genetic which could create a pathological functional system can exist in principle as well. As was noted earlier, such sources of information could obviously include programs of the normally functioning organism and emotion-behavior programs at the level of temperament which fall out of integrated control by the body as a whole, becoming in this case pathologically autonomous systems. Existence of mental diseases and neurosis implies the possibility of formation of the information nucleus of the pathological system at the mental, specifically human, personality level. These issues have a direct bearing on the problem of the unconscious and on psychotherapy.

When we interpret disease as a functional system, we are able to raise the issue as to the nature of mental phenomena in the presence of which, from this standpoint, some part of the ensemble of the functional systems of the brain and personality are "separated," "broken" away (or new systems are formed on the basis of information specific to this ensemble), coupled with creation of a closed or, to one extent or another, an autonomous system, in a new way. Different forms of mental automatism, split personality phenomena, and so on might correspond to this at the psychopathological level. Apparently, many disease manifestations represent competing normal and pathological systems.

In conclusion I would like to emphasize the basic premise that disease, viewed as a system, has the capability for self-maintenance and active adaptation. The problems of pathomorphosis are apparently closely associated with this adaptive feature of pathological systems. The therapeutic strategy based on the interpretation of disease as a system must account for the adaptive capabilities of disease.

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THE REHABILITATION CONCEPTION AND MODERN CLINICAL MEDICINE

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[Article by M. M. Kabanov]

[Text] The roots of the rehabilitational direction in medicine extend deep into history. However, the modern definition of this complex, developing conception is commonly believed to have originated during World War II.

The essence of rehabilitation is defined differently today; many specialists interpret it as a set of various influences and measures usually implemented after termination of appropriate therapy. In internal medicine (including nervous diseases), rehabilitation is interpreted for practical purposes as various procedures (gymnastics, massage, early release from bed rest following an infarction, insult, or surgery, and so on) in a sense attached to "conventional" therapy and having the purpose of optimizing it.\* Naturally, traumatologists and orthopedists devote a great deal of attention to prosthetics in rehabilitation measures, physiotherapists place emphasis on physical therapy, and psychiatrists turn their attention to vocational therapy and psychotherapy. Representatives of all specialties define rehabilitation as sensible adaptation of the patient (invalid) to home and work. Expert certification of working ability has great significance in this case. When we take account of the specific features of the particular medical specialty, none of these premises elicit debate, but when taken out of "context" they unjustifiably constrict the goals and tasks of rehabilitation, which should be interpreted much more broadly, mainly in the form of a complex system.

An extensive, thorough definition of rehabilitation was given at the Ninth Conference of Socialist Ministers of Public Health and Social Welfare (Prague, 1967), a resolution of which defines rehabilitation as a system of various measures (governmental, economic, medical, psychological, social, and others) aimed not only at optimum adaptation of the patient (invalid) to life and labor but also at preventing some particular defect (disability). This

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\*Of course, there are other definitions as well, for example those encountered in the works of T. D. Demidenko and her colleagues.

preventive aspect of rehabilitation is often ignored, which does harm to the theory and practice of rehabilitation.

I have written many times that rehabilitation is not just a goal in itself--restoration of the patient's personal and social status (complete or partial); it is not only a process accelerating medical recovery, a process having its own neurophysiological and psychological parameters; it is also a way of approaching the sick person characterized by four fundamental premises. I will simply list them here. They include, first, the principle of partnership between the physician and patient in attaining the objectives of rehabilitation; then there is the principle of diversity (different levels) of efforts, influences, and measures directed at various spheres of the life of the person undergoing rehabilitation--family, work, social, and leisure, and at changing his attitude toward himself and his disease; next comes the principle of the unity of biological (medicinal therapy, physiotherapy, and so on) and psychosocial (psychotherapy, vocational therapy, and so on) methods of influence; finally there is the principle of stage-by-stage influence--that is, transition of one form of influence or a measure into another (I. P. Pavlov had turned his attention to this premise, calling it fundamental to any type of human activity). When rehabilitation is defined in this way, therapy must be directed not only at eliminating pathological manifestations but also at producing qualities in the patient helping him to adapt as best as possible to the environment. It is clear from the fundamental premises presented here that only a human can be rehabilitated while, as we know, both man and animals can be treated.

Rehabilitation defined as a conception requiring synthesis (integration) of different forms of knowledge (medical, psychological, social) opens up new avenues for reexamination of a number of theoretical premises in medicine. This conception is directly related with the fundamental premises of human ecology (A. D. Ado) and permits us to examine, in a new way, the most important problems in the relationship between that which is biological and that which is social in the origin of diseases, in their development, in their prevention, and in their treatment. I (M. M. Kabanov, 1974) define rehabilitation as a dynamic system of mutually associated components (medical, psychological, social) aimed at attaining a final goal (restoration of the status of the personality) by a special method, the essence of which was presented above.

When we examine rehabilitation from the standpoint of the systems approach, we gain the possibility for understanding it correctly as a system of activity, the participants of which include the individual (who is himself an open system) and the biological and social environment surrounding him, in their interaction. The end goal (concrete result) of rehabilitation is the system-forming factor. Within the rehabilitation system, we should distinguish among subsystems together with their basic elements. Thus the basic elements of the clinical-biological subsystem are embraced by concepts such as homeostasis, adaptation and compensation; the psychosocial subsystem includes the attitudes and mutual relationships of the patients and the surrounding social environment. Were we to use the classification of systems suggested

by P. I. Kal', rehabilitation could apparently be interpreted as both a dynamic and a conceptual system. Application of the systems approach to rehabilitation permits us to avoid viewing the relationship between that which is social and that which is biological in human pathology (and not in pathology alone), a highly important issue in medicine, as one of alternatives, and it reveals the way for surmounting this tendency. In rehabilitation, we must account for a tremendous quantity of medical, psychological, and social data (indices). It is extremely difficult to reveal (record) the indices, and especially to establish their mutual relationships and find their "informative correlations." An important "tool" that helps us to account for and measure numerous indices and establish correlations among them in the process of rehabilitation is the so-called rehabilitation charts and rating scales. The encoding performed by means of these tools makes it possible to process a tremendous mass of data with the help of various mathematical methods and specially developed computer programs. Mathematical statistical analysis performed from the positions of the systems approach opens up new possibilities for scientific study of the interrelationships of a large number of components in the rehabilitation system and creates the preconditions for adequate statement of concrete tasks and attainment of the greatest "efficiency" in their completion (G. V. Burkovskiy et al.).

The rehabilitation conception is beginning to have an influence on development of a new understanding of the clinical pattern of different diseases. It should be stated that the concept "clinical pattern" itself is dynamic, constantly changing in its development. That which had not pertained to it yesterday pertains today with sufficient obviousness. For example the concept "internal picture of disease" (R. A. Luriya) is now becoming part of the content of the term "clinical pattern," and there was once a time when clinical blood analysis, customary today, was not included in this concept. The clinical pattern of many diseases has experienced significant change in the last two or three decades. Arisal of many "diffuse," atypical forms of a number of diseases, particularly psychoneural and cardiovascular, bringing them closer to states which are commonly called "borderline," can be explained by various causes. They include so-called medicinal pathomorphosis, the child and adolescent acceleration factor, and demographic changes in the population (enlargement of the number of elderly and, as a consequence of this, growth in the number of diseases typical of elderly and senile age, together with the atypical course inherent to many diseases at this age).

Psychosocial factors,\* which lead in a number of cases to emotional stress, doubtlessly influence the growth of forms of human psychoneural pathology such as neuroses, so-called psychosomatic diseases, and so on, the clinical pattern of which is complex and fails to fit within the framework of

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\*"Factors having an influence on health, public health services, and the society's welfare, forming out of the psychology of the individual and the structure and functions of social groups" are implied (from a report of the WHO general director at the 57th Executive Committee Session, EB 57/22, 20 November 1975).

traditional medical ideas, oriented for the most part on the biological model of illness. "Clinical pattern" is not only a dynamic but also a systemic concept. As with the principle of historicism, the systems principle is a fundamental premise of Marxist dialectics (V. P. Kuz'min) and, on this basis, we must once again emphasize that that which had been referred to as part of the concept of the "clinical pattern" of particular diseases is now becoming insufficient. The purely biological model of disease, which is essentially reductionistic, is now being replaced by a "mixed" model where biological and social factors (psychosocial factors more precisely) supplement one another, intertwining differently in different stages of the course of a given disease. Ye. K. Krasnushkin's old statement that man has not a single organ function that is not wrapped in social clothing integral with it acquires a new ring in connection with the rehabilitation conception. For example the clinical pattern of mental diseases presently includes not only the description of certain "traditional" psychopathological phenomena (hallucinations, delirium, depression, dementia, and others) but also new often more-complex psychopathological (and often prepsychopathological) characteristics such as "deviant behavior," character accentuation, institutionalism, and others which cannot be understood correctly with the assistance of just clinical (medical) biological categories. In addition to the latter, we need other categories--clinical (medical) psychological and sociomedical. It stands to reason that the clinical biological aspects of research on mental diseases have hardly been exhausted, and that they require further study. But research conducted from a systems standpoint with a consideration for the greatest informativeness of the "crossroads" of different sciences (for example genetic research making use of not only biochemical but also pathopsychological methods, research on the role of ecological factors in various diseases with a consideration for biological rhythms, and so on) appears especially promising in this area as well. To put it more briefly, we need the broadest research that would deepen our understanding of human nature. But clinical psychological and sociomedical research aimed to a greater extent not at the nature but rather at the essence of man is no less important. "The growing sociologization of the life of modern man," A. A. Pokrovskiy and G. I. Tsaregorodtsev write, "is defining the nature of his pathology to an increasingly greater degree."

The conception of rehabilitation, which presupposes an integrated approach to the sick person and utilization of all of the parameters of his vital activities, provides a new impetus to development of the clinical direction on the basis of rich experience and the material of numerous clinical studies conducted in the past few decades. The issue of functional diagnosis (raised for the first time in domestic literature back in the 1930's by M. P. Konchalovskiy) is being raised again in a broader aspect in connection with the objectives of rehabilitation. Functional diagnosis, which accompanies the analytical-synthetic activity of the physician, consists of three parts ("sides of a triangular file" to put it metaphorically)--nosological, psychological, and social. When taking the rehabilitation approach toward a patient, the physician must know not only the "name" of the disease (nosological and

syndromological diagnosis) but also the person (the type of personality) in whom this disease arises and the environment (the social microenvironment is implied) in which it arises.\* Functional diagnosis is what answers these questions (V. M. Volovik). Directed psychosociotherapeutic influence upon the patient, which is now enjoying acceptance in the internal disease clinic as well, would be purposeless and even chaotic if we do not take account of the corresponding "sides of functional diagnosis."\*\* Modern forms of psychotherapy cannot be used adequately without addressing them to the patient's personality. The same can also be said for modern forms of sociotherapy,\*\* among which various forms of organizing the therapeutic environment and of work with a group of patients are acquiring the greatest significance today. It stands to reason that the nosological "side" of functional diagnosis also demands a different relationship toward it, a closer "alloy" with its other "sides." Wing et al. believed that the action of medicinal preparations (psychotropic drugs) depends on the conditions in which the particular patient is located (in a hospital, in a day hospital, at home). From my point of view functional diagnosis is true clinical diagnosis in light of our present definition of the clinical patterns of the most diverse diseases, associated not only with nosos (and pathos) but also with specific features of the personality and the environment. The need for qualified functional diagnosis requires cooperative work by the physician and the medical psychologist; we might also dream about medical sociology, but this is a matter for the future. For the moment, we do not have sociologists in the clinic, and we will have to get by with just psychologists, fundamentally altering their style of work.\*\*\*\* "Analysis of mental functions, processes, and states taken in isolation can no longer satisfy the needs of developing theory," say A. Leont'yev, B. Lomov, and V. Kuz'min, and to this we add that it cannot satisfy not only theory but also practice--the practice of rehabilitation, the practice of making (together with a physician) a functional diagnosis.

Discussing the influence of the ideas of rehabilitation on various aspects of medical theory and practice, we should once again emphasize the significance of this conception to treatment of pathological states. We would have

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\*It may be argued that the physician has always accounted for these circumstances (especially a good physician). But the entire matter lies with where the accent is placed. The rehabilitation approach to a patient (with any diagnosis whatsoever) requires not just declaration of the existence of these circumstances but also the real act of approaching them.

\*\*Biological treatment can also be "chaotic," leading to unfavorable consequences; it would be sufficient to recall countless use of the number of psychotropic preparations (aminazin for example), or combinations of medicinal agents "by eye."

\*\*\*This term is very indefinite and is subjected to extremely broad interpretation. A close mutual relationship exists between psychotherapy and sociotherapy, and it is sometimes difficult to draw a line between them.

\*\*\*\* The activities of a medical psychologist in a practical institution must be closely associated with therapeutic and restorative work; otherwise as a rule it loses its meaning and fails to get the respect it deserves from treating physicians.

to agree with scientists suggesting that "rehabilitation" is a broad concept, including therapy in its generally accepted definition. Querido validly points out that extremely arbitrary boundaries, created, in his words, "to facilitate our thinking," exist between prevention, therapy, and rehabilitation.

I had noted earlier that rehabilitation differs from "conventional therapy" in that qualities helping the patient to adapt in optimum fashion to the social environment must be developed through the joint efforts of the physician (and all personnel) and the patient (and his surroundings, chiefly the family). Therapy means that which is directed for the greater part at the body, at the problem at hand, at the present, while rehabilitation is addressed more to the personality and in a sense is aimed at the future. An idea of the stages of rehabilitation can be given by the division I made of this dynamic process in 1968 into three successive stages--restorative therapy, readaptation, and rehabilitation in the direct sense of this word. Subdivision of rehabilitation into three stages had been proposed earlier by WHO experts\* and is broadly accepted in world practice (G. S. Yumashev, K. Renker). But restorative therapy should be interpreted not only as a means for readaptation\*\* and rehabilitation but also as the beginning of these complex processes. The objectives of rehabilitation as well as its forms and methods change depending on the stage. While in the first stage (restorative therapy) the objective is prevention of a defect, institutionalism, and disability (or their compensation), in subsequent stages it is adaptation of the individual to life and labor, sensible placement of the patient at home and at work, and creation of a favorable psychological and social microenvironment, which of course also serves the objectives of prevention but is now "secondary" or "tertiary." The forms of influence are also diverse, varying from initial active biological therapy, which is gradually substituted by maintenance pharmacotherapy (for example using psychotropic drugs) or which is generally eliminated, to various methods of "milieu therapy," psychotherapy, vocational therapy, and occupational therapy, the role of which grows in subsequent stages. It stands to reason that the "level" of rehabilitation can also vary depending on the severity of the disease, the specific features of the clinical symptoms, and the specific features of the patient's personality and the social conditions (presence of a family, the nature of mutual relationships, and so on). The variation we see in the way many specialists interpret rehabilitation can be explained by failure to account for the stages of this process and absence of common theoretical principles. Rehabilitation is not simply optimization of therapy, as is sometimes suggested, at least because its measures are

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\*"Meditsinskaya, professional'naya i sotsial'naya reabilitatsiya" (Medical, Professional, and Social Rehabilitation), 1958. I now suggest that all stages of rehabilitation must bear both a medical and a psychosocial "coloration"; only the degree to which medical and psychosocial factors are expressed in different stages changes.

\*\*It should be noted that only this term is employed in French-speaking countries; the term "rehabilitation" is not used.

directed not only at the patient himself but also at his surroundings (primarily at the family). Hence follows the tremendous significance of group psychotherapy,\* family therapy, and milieu therapy.

Thus the "therapy" concept is supplemented by new content in light of the rehabilitation conception. As long ago as in the 1930's V. N. Myasishchev identified psychotherapy, which has a tremendous role in the complex of rehabilitational influences, closely with medical pedagogics. Now that new data have arisen\*\* attesting to concealed human capabilities--capabilities for making greater use of mental reserves--the concept "psychological defenses" (F. V. Bassin) is also perceived in a new way, requiring methodological development with the goal of making use of it in restorative therapy and in subsequent stages of rehabilitation.

The premises presented here indicate that the tactics and strategy of the physician in diagnosis and therapy are changing. In light of the rehabilitation conception, these premises have, as was noted earlier, a historical aspect of development and can be satisfied properly if reliance is placed upon the systems principle. This is that very alteration of medical thinking for which our doctors have long been appealing. But in order to become real, this "alteration of thinking" requires two "nutrient mediums." First it would require creation of other forms for training specialists--physicians, medical psychologists, and social workers. It would be especially important in this case to note the need for coordinated work on the part of specialists of different profiles. Using the commonly accepted terminology, in this team work, which is conducted specially in the first stages of rehabilitation under the general guidance of the physician, we must comply with the "principle of partnership," presupposing retention of the given specialist's own physician (own style) in his communication with the patient. Let me emphasize that we would not be able to do real work in rehabilitation without appropriately training and retraining the personnel (physicians, psychologists, nurses).

But appropriate organization of medical care is a no less important factor, one without which we cannot have progress in anything, especially rehabilitation. Specifically speaking, the trend toward development of medical service "in the rehabilitation direction" has long been in existence, especially in our country. This trend has been acquiring new forms in the last few years in connection with expansion of the rehabilitation direction. As an example our understanding of the place and significance of day hospitals and other institutions of "partial hospitalization" is

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\*I make a distinction between "group therapy" (utilization of group influence in all work with patients, for example in therapeutic physical exercises) from "group psychotherapy," which is also close to "family psychotherapy." However, this distinction is often very arbitrary.

\*\*See for example the method of the Bulgarian scientist G. Lozanov.



growing (V. M. Volovik et al.). Psychotherapeutic offices are being organized at general polyclinics, and mental hygiene offices providing consultative assistance, particularly in relation to family problems, are being created in some cities (for example in Moscow and Vil'nyus). In other places (for example in Riga and Tartu) clubs organized for persons who had suffered particular psychoneural diseases have been functioning successfully for a long time. The concluding stage of mental patient rehabilitation is characterized by development of so-called industrial rehabilitation (Ye. D. Krasik et al.; A. V. Grosman; A. I. Gurevich; A. Ye. Lifshits and Yu. N. Arzamastsev; L. I. Kruglova)--that is, work placement of patients coupled with creation of a favorable "psychological climate" for them at conventional industrial enterprises.

The issue of organizing specialized communal residences (boarding houses) for patients suffering severe forms of chronic diseases and unable to live with relatives for one reason or another is very important; the experience of such residences has already been described in our literature (R. Ya. Mar'yanchik; A. S. Gladyshev). Alteration of the activity of social welfare hospitals also deserves much attention (V. P. Belov and V. A. Galkin). But what is most important in this complex organizational work is to achieve clear coordination among all levels of the numerous services subordinated to different departments.

The socialist nature of our society, growth in its welfare and culture, and the increasingly greater attention being devoted here in our country to the individual, are a guarantee of further successes in the complex work of fighting for the individual's physical and mental health.

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ANALYSIS OF COCHLEOVESTIBULAR CORRELATIONS ACCOMPANYING THE EFFECTS OF NOISE AND VIBRATION

Moscow VESTNIK OTORINOLARINGOLOGII in Russian No 2, 1978 pp 17-20

[Article by Prof V. Ye. Ostapkovich and Cand Med Sci I. M. Korol', Scientific Research Institute of Labor Hygiene and Occupational Diseases, USSR Academy of Medical Sciences, Moscow]

[Text] The effect of noise and vibration on the acoustic analyzer has been examined by a large number of authors, but little work has been done on the action of these factors on the vestibular analyzer and on mutual cochleo-vestibular relationships accompanying the effects of noise and vibration.

That such relationships exist can be judged from the presence of the cochleo-vestibular anastomosis, a bundle of nerve fibers. These fibers are interpreted as a link between vestibular and cochlear nerves. Some scientists believe that they are either part of the cochlear nerves (10) or a special deviating cochlear bundle (9,12-14). It has also been hypothesized that they travel together with the cochlear nerve but from vestibular receptors (1,6,7,11). Min'kovskiy et al. (2) concluded on the basis of electronystagmographic research performed on patients suffering cochlear neuritis by the cupulometry method that parallelism exists between degenerative changes in the cochlear and vestibular branches of cranial nerve VIII.

Basing ourselves on these anatophysiological data and clinical observations, we analyzed the correlations of all characteristics of nystagmus coupled with impairment of hearing at individual frequencies, and we studied nystagmic and cochlear intercorrelations.

The raw material consisted of many years of observations of workers from 20 to 50 years old subjected to the effects of noise and vibration, basically the results of audiometric and vestibulometric analyses. Correlation analysis was performed with a Minsk-32 electronic computer.

Here are two examples of our observations of patients suffering occupationally associated hearing impairment illustrating extreme deviation in the degree to which vestibulocochlear disorders are expressed.

Patient S., 42 years old, has worked in the forging shop continuously for 23 years. He experiences the effects of pulsed noise having an intensity of 120-135 db. He complains of noise in the ears and bad hearing, which arose during his 13th year of work. His therapeutic, neurological, ophthalmological, and otorhinolaryngological status is uneventful. Threshold audiometry results: Symmetrical hearing impairment of both ears is, in relation to the type of sound perception, 45 db at 125 Hz, 55 db at 500 Hz, 65 db at 2,000 Hz and 85 db at 4,000 Hz. Electronystagmographic data: When rotation occurs to the right, the duration of nystagmus is 10 sec, the number of cycles is 18, the total amplitude is 57°, and the rate of the slow phase is 30° per second; when rotation occurs to the left, the duration of nystagmus is 13 sec, the number of cycles is 30, total amplitude is 94°, and the rate of the slow phase is 33.5° per second.

Rhythm disturbance and irregular nystagmus amplitude are observed. Nystagmus with a duration of 98 sec was recorded in response to warming of the left ear, while individual nystagmic "bursts" (several nystagmic cycles following one another) in an interval up to 80 sec from the moment of their first arisal were revealed in response to warming of the right ear.

Treatment by B vitamins, ATP intramuscularly, and general fortifying agents was conducted several times. Later the patient was transferred to work not involving intense noise.

This example indicates a considerably progressed form of impaired hearing combined with inhibition of vestibular function. Parallelism of degenerative changes in the acoustic and vestibular analyzers was noted in this observation

During this same time we had another worker of this shop under our observation--patient M., 48 years old, with a work career of 18 years in the same conditions experienced by patient S.. His hearing decline was approximately the same, while the duration of postrotation nystagmus was 30-35 sec, that of caloric nystagmus was 50 sec, and the frequency, the amplitude, and the rate of the slow phase indicated rhythmic nystagmus with moderate characteristics.

Observation of the dynamics of work-related hearing impairments established correlation in the losses of hearing at individual frequencies. The progressive decline in hearing gradually affects the zone of middle and low frequencies (3-5, 8, 15, etc.). We also obtained a confirmation of the presence of cochlear intercorrelations not only from dynamic observations but also with the help of correlation analysis. For the group of workers subjected to the effects of noise and having suprathreshold audiometry data indicating different levels of affliction of the acoustic analyzer, the correlation coefficient ( $r$ ) between the thresholds of sound perception at 500 and 2,000 Hz was  $0.82 \pm 0.04$  ( $P < 0.001$ ), between 500 and 4,000 Hz it was  $0.59 \pm 0.08$  ( $P < 0.001$ ), and between 2,000 and 4,000 Hz it was  $0.60 \pm 0.08$  ( $P < 0.001$ ), which attests to adequate precision of our mathematical method of analysis. In contrast to this, cochleovestibular correlations were found to be less significant. Statistically significant values ( $P < 0.05$ )

indicated presence of negative correlation between the total number of cycles of postrotation nystagmus and decline of hearing at 500 Hz among persons subjected to the effects of noise and vibration. Decline in hearing at 500 Hz was accompanied by a decrease in the number of cycles of postrotation nystagmus. The probability of this correlation was not significant for 250 Hz, though it was close to significant ( $0.05 < P < 0.1$ ).

Correlation was revealed between hearing decline at 500 and 2,000 Hz and the frequency of nystagmus in the first 5-second interval for the group of persons subjected to the effects of noise alone ( $r = -0.27$ ,  $P < 0.05$ ). This means that the greater the decline in hearing is within the range of indicated frequencies, the fewer is the number of cycles per second in the response of the vestibular analyzer to stimulation of the labyrinths.

Different nystagmic indices reflect the same process--a reaction to stimulation of the labyrinths, and in this connection it would be natural to hypothesize that a relationship exists between these indices. We can also hypothetically suggest presence of correlation between postrotation and caloric reactions, inasmuch as the latter are the product of the same functional systems of the vestibular analyzer.

In a number of cases the electronystagmograms were so demonstrative that one could be persuaded visually of matching changes in frequency, amplitude, and the rate of the slow phase. In addition we observed cases of disturbed rhythm, reduction in amplitude of individual cycles, and changes in the slope of the line representing the slow component of nystagmus with respect to the time axis. Sometimes in the presence of rhythm disturbances the mean amplitude and the rate of the slow phase experienced minor changes and, on the other hand, individual "deviant" cycles with dramatically differing characteristics occurred.

The duration of this segment is the easiest index to determine, and this is why there is so much interest in using it for occupational selection and in periodic medical examinations, when the need arises for examining large groups of persons.

The probability of the relationship of nystagmus duration to all other indices is so high ( $P < 0.001$ ) that the possibility of using an assessment of the nystagmus reaction as one of the criteria cannot be doubted. The extremely high coefficients of correlation between the duration and total number of nystagmic cycles (0.92 and 0.93) as well as total amplitude (0.84 and 0.88) confirm the diagnostic significance of this index.

The mutual correlation of the total number of nystagmic cycles for the group of people subjected to noise and for the group of people subjected to noise and vibration is characterized by the following correlation coefficients respectively: Correlation with amplitude in the first 5-second interval-- $0.55 \pm 0.09$  and  $0.73 \pm 0.07$ ; correlation with total amplitude-- $0.83 \pm 0.04$  and  $0.91 \pm 0.03$ ; correlation with the rate of the slow component-- $0.39 \pm 0.11$  and

0.78±0.06. The highest correlation was observed for amplitude and the rate of the slow phase, functionally associated with the time factor; the coefficients were 0.86±0.03 and 0.96±0.01 respectively.

However, significant values were also obtained for total amplitude--0.55±0.09 and 0.89±0.03. The high significance of the correlations among most of the analyzed indices indicates the possibility for using each of them to characterize the nystagmic process.

The results of comparing the latent times of the caloric reactions were highly unexpected. It was found that latent time correlates not only with indices of caloric nystagmus for persons subjected to noise but also with the indices of postrotation nystagmus. The highest correlation was revealed with the rate of the slow component. It is entirely possible that latent time and the rate of the slow component reflect the same qualities of the vestibular analyzer, which could be interpreted conditionally as reactivity.

Correlation between latent time and other indices of caloric nystagmus persists in the group subjected to noise and vibration, but among all indices of postrotation nystagmus, only duration was found to have a statistically significant correlation coefficient ( $r=0.37\pm0.13$ ;  $t=2.84$ ;  $P<0.01$ ).

The correlations between the characteristics of postrotation nystagmus and caloric nystagmus are of considerable interest inasmuch as the stimulation method and the quantitative values of the responding reaction differ dramatically in this case. Examinations showed that cases difficult to explain are encountered, in which rotation elicits postrotation nystagmus of short duration while calorization produces prolonged nystagmus (up to 100 sec). Moreover in some cases absence of nystagmus following rotation was combined with arousal of a normal nystagmic reaction following warming. Such cases cannot be explained from the standpoint of hydrodynamic theory, since rotation was a more-intense stimulus than was warming.

The examples presented above indicate that the data of simple clinical observations do not always permit us to establish some sort of correlation between caloric and postrotation nystagmus, since the data are sometimes highly contradictory. At the same time the results of analysis of correlations were unexpected: Positive correlation was revealed between the indices of postrotation and caloric nystagmus for the group of persons subjected to noise (Table 1).

Despite the qualitative heterogeneity of the caloric and rotation stimuli, in most cases the somatic reaction of the vestibular analyzer had its own individual parameters mutually associated in quantitative respects with the indices of nystagmus. It is thus obvious that this "internal" order of the characteristics of the nystagmic reaction indicates strict coordination of the functions of all morphological structures of the vestibular complex, to include the vestibular nucleus and higher levels.

Table 1. Correlation ( $r$ ) Between Postrotation and Caloric Nystagmus for Patients Subjected to Noise ( $n=64$ ;  $r \geq 0.25$  at  $P < 0.05$ ).

(1) Калорический нистагм	(2) Послевращательный нистагм					
	длитель- ность (3)	частота (4)	сумма циклов (5)	амплитуда (6)	тотальная амплитуда (7)	СМФ (8)
(9) Латентный период	0,41	0,33	0,45	0,42	0,45	0,35
(3) Длительность	0,62	0,38	0,65	0,41	0,55	0,25
(4) Частота	0,52	0,46	0,63	0,33	0,42	0,18
(5) Сумма циклов	0,61	0,40	0,69	0,32	0,48	0,16
(6) Амплитуда	0,51	0,31	0,53	0,27	0,42	0,17
(7) Тотальная амплитуда	0,58	0,30	0,60	0,27	0,46	0,15
(8) СМФ	0,47	0,37	0,50	0,36	0,46	0,29

Key:

- |                           |                           |
|---------------------------|---------------------------|
| 1. Caloric nystagmus      | 6. Amplitude              |
| 2. Postrotation nystagmus | 7. Total amplitude        |
| 3. Duration               | 8. Rate of the slow phase |
| 4. Frequency              | 9. Latent time            |
| 5. Total cycles           |                           |

Table 2. Correlation ( $r$ ) Between Postrotation and Caloric Nystagmus for Patients Subjected to Noise and Vibration ( $n=64$ ;  $r \geq 0.29$  at  $P < 0.05$ ).

(1) Калорический нистагм	(2) Послевращательный нистагм					
	длитель- ность (3)	частота (4)	сумма циклов (5)	амплитуда (6)	тотальная амплитуда (7)	СМФ (8)
(9) Латентный период	0,37	0,18	0,23	0,19	0,26	0,13
(3) Длительность	0,36	0,13	0,24	0,15	0,28	0,12
(4) Частота	0,35	0,13	0,30	0,14	0,28	0,15
(5) Сумма циклов	0,32	0,09	0,24	0,06	0,22	0,05
(6) Амплитуда	0,34	0,02	0,25	0,13	0,28	0,12
(7) Тотальная амплитуда	0,34	0,06	0,24	0,14	0,29	0,12
(8) СМФ	0,39	0,06	0,29	0,19	0,33	0,20

Key:

[See Key, Table 1]

The low coefficients of correlation between the rate of the slow phase of postrotation and caloric nystagmus and its frequency-amplitude characteristic is obviously explained by the great variability of the rate of the slow phase. However, the rates of the slow component correlate between each other in the two reactions, confirming the individuality of nystagmus characteristics. The fact that the examples of individual clinical observations presented earlier fail to fit with the described correlations more likely indicates change in mutual relationships arising as a result of pathological processes. But as we can see from the general correlations for the entire group of subjects, they are not typical of persons subjected to noise.

The coefficients of correlation between the indices of postrotation and caloric nystagmus for persons subjected simultaneously to noise and vibration are presented in Table 2.

It is obvious from Table 2 that in most cases the nystagmus indices for the different reactions do not correlate among each other, and that normal mutual relationships are disturbed. This should probably be interpreted as disturbance of the coordinating influences of the higher divisions of the vestibular analyzer. To an equal degree, the revealed features might also be the product of the peripheral division, which generates impulses differently in response to stimulation of the labyrinths by adequate and inadequate stimuli. For the group of patients subjected to noise, these disturbances were singular and they did not manifest themselves in the correlations of most subjects; this is why statistically significant values of  $r$  were obtained for almost all indices. For persons subjected to noise and vibration simultaneously, we observe more-frequent disturbances of the mutual relationships between nystagmus indices, reflecting themselves in the values of the correlation coefficients.

Application of the correlation method documented absence of correlation between most indices of vestibular nystagmus and impairment of hearing at individual frequencies, indicating that pathological changes occurring in the cochlea in response to intense noise are isolated. Perception of 500 Hz sound is an exception, in which case weak correlation was revealed with the total quantity of nystagmic cycles and total amplitude among patients subjected to noise and vibration.

Analysis of the correlations also demonstrated presence of individual specificity of the nystagmic reaction, irrespective of whether an adequate or a temperature stimulus was employed. The quantitative values of the indices of postrotation and caloric nystagmus for the same subjects exhibited direct correlation.

For the group of persons subjected to vibration and noise, the mutual relationships vary, since correlation between the indices of postrotation and caloric nystagmus is disturbed. Clinical observations established arisal of dysrhythmia and variable-amplitude nystagmus. The effect of vibration can be hypothesized as the cause behind arisal of such phenomena.

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DRY COMBINED AEROSOL THERAPY FOR WORKERS FREQUENTLY SUFFERING ACUTE RESPIRATORY DISEASES AND ANGINA

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[Article by Dr Med Sci N. V. Zberovskaya, Cand Med Sci T. G. Minyayeva, Cand Med Sci K. I. Savitskaya, L. B. Fedotova, and V. V. Osinyagova, Department of Otorhinolaryngology, Moscow Oblast Scientific Research Clinical Institute imeni M. F. Vladimirskiy]

[Text] Acute respiratory diseases and angina contribute the greatest amount to the number of cases of illness coupled with temporary loss of working ability at industrial enterprises. Each year 10-20 out of 100 persons are stricken with acute respiratory diseases while 6-10 suffer angina (2,4,7). A large number of days of incapacitation are the product of complications following these diseases--bronchitis, pneumonia, and so on.

Among the causes eliciting disturbance of the protective function, the main ones are various pathological changes in the pharynx, nose, and accessory sinuses. Chronic diseases of the ear, nose, and throat have been revealed among 70-100 percent of the workers that are frequently stricken with acute respiratory diseases and angina (5,8,11).

Attempting to develop a simple mass treatment method to be applied to the upper respiratory passages of workers in industrial enterprises frequently suffering acute respiratory diseases and angina, we employed intranasal insufflation of dry antibacterial and hyposensitizing agents (dry combined aerosols). Dry aerosol therapy was preferred over other treatment methods because the medicinal powder, when introduced into the nasal cavity, penetrated directly to the focus of affliction, being sucked into the same channels as pathogenic microbial flora (1,10).

In contrast to moist aerosol therapy, dry aerosol therapy did not require creation of special inhalators, and it can be performed with a minimum time outlay by individuals performing the sanitation procedures themselves.

Our experimental research on the pathways followed by antibiotics and sulfanilamides insufflated in the form of a dry combined powder in the nasal cavity also provided the grounds for using this method for introducing medicinal



agents. The research consisted of the following: Ten rabbits were subjected to one-time intranasal insufflation of a powder consisting of norsulfazol (0.25 gm), oxacillin (0.025 gm), dimedrol (0.025 gm), and ephedrine (0.012 gm). The rabbits were killed at different intervals, and the concentration of antibacterial preparations in the blood, in the mucous membrane of the nasal cavity, in the tonsil, and in lymph nodes of the neck was determined. The antibiotic concentration was determined by the serial dilution method.

Forty analyses were performed in all, indicating that oxacillin and norsulfazol are retained in the nasal cavity for up to 6 hours at concentrations from 22 to 0.1  $\mu\text{g/ml}$ . One hour after insufflation the antibacterial preparations were determined in blood (0.2<sup>1</sup>-1.2  $\mu\text{g/ml}$ ) in lymph nodes of the neck (0.02-3.0  $\mu\text{g/ml}$ ), and at especially high concentrations in tonsil tissue (6.5  $\mu\text{g/ml}$ ). Later, a therapeutic concentration of oxacillin and norsulfazol persisted in the palatine tonsils and blood for up to 3 hours following insufflation, and up to 6 hours in lymph nodes of the neck.

Thus our experimental research showed that oxacillin and norsulfazol insufflated into the nasal cavity saturates the tonsils and local lymph nodes at large concentration; they are deposited in the latter for not more than 6 hours.

If we consider that patients suffering chronic inflammatory processes in the upper respiratory passages simultaneously have foci of latent infection in local lymph nodes that are difficult to treat by conventional methods (3,6), it could be hypothesized that antibacterial preparations penetrating into lymph nodes of the neck in sufficient concentration following intranasal insufflation should have a therapeutic action on chronic foci of infection in the vicinity of ear, nose, and throat organs.

We used a combined powder of the same composition studied in the rabbit experiment for mass treatment of workers in industrial enterprises frequently suffering acute respiratory diseases and angina.

Our bacteriological analyses of the microflora of the upper respiratory passages and our determination of its sensitivity to antibiotics and sulfanilamide in 100 frequently ill workers served as the grounds for using an aerosol of precisely this composition. These analyses showed that streptococci (hemolytic and vegetating), enterococci, and staphylococci are revealed in most cases. Sensitivity of the flora was highest to erythromycin and oleandomycin. It was found to be two times lower in relation to oxacillin. The flora was practically nonsensitive to norsulfazol. Nevertheless we preferred other antibiotics, namely a combination of oxacillin and norsulfazol, because they were found to be clearly synergistic in their effect on isolated microorganisms. Thus while there was a zone of inhibition of microbial growth of only 10-15 mm about standard discs bearing oxacillin, the diameter of the growth inhibition zone attained 50-60 mm about discs saturated with oxacillin and norsulfazol (the zones of inhibition of microbial growth were two times smaller when erythromycin and oleandomycin were used). In addition to these data, we took account of a report that norsulfazol promotes prolongation of the action of oxacillin in the body (12).

Insufflation was first performed either with a conventional powder sprayer, or by means of inhalation of the combined powder through the nose from paper envelope, or with individual microinsufflators (9). Later, we (I. M. Kondrashov and N. V. Zberovskaya, Efficiency Proposal No 275, 1976) developed an insufflator with a dosing device which, after being loaded once (with up to 100 doses), could quickly dispense identical portions of the medicinal powder and be used for insufflation right at the workplace of persons undergoing treatment. The insufflator was supplied with removable tips to insure sterility. Using this device, the shop nurse was able to perform intranasal insufflation of the dry aerosol on 50-60 workers within 1 hour right at their workplaces.

In order to clarify the length of time that the antibacterial preparation is deposited in the body following one-time intranasal insufflation of the combined powder, the time of its elimination by the kidneys of 31 persons was determined (67 urine analyses were performed). The research established that the antibacterial preparations (oxacillin and norsulfazol) were eliminated from the body at sufficiently high concentration in 24-48 hours, and at a lower concentration for up to 72 hours. This research showed that it would be enough to insufflate the combined medicinal powder into the nasal cavity just once a day to maintain a constant therapeutic concentration of the antibacterial preparations in the body.

Considering that acute respiratory diseases and angina are usually observed in the spring and fall, we conducted preventive aerosol therapy in the form of two 10-day courses--in February and in September. Beginning with 1970, such aerosol therapy was performed at six enterprises of Moscow Oblast. Aerosol therapy was employed for 2 years at each of these enterprises: In 1970-1975 at the Novo-Noginsk Textile Factory, in 1973-1974 at the Shchelkova Poultry Factory and at the Pamyat' Il'icha Kolkhoz, and in 1975-1976 at the Gigant and Krasnyy Stroitel' industrial enterprises in Voskresensk and at the Orekhovo-Zuyeva Cotton Combine. In all, 525 workers suffering various chronic diseases of the ears, nose, and throat and often suffering acute respiratory diseases, angina, bronchitis, and pneumonia were subjected to treatment. Among ear, nose, and throat diseases, chronic tonsillitis was revealed in 41.8 percent of the observations; chronic diseases of the nose and the accessory sinuses was revealed in 19.1 percent of the observations; the remaining 39.1 percent consisted of chronic tonsillitis combined with chronic pharyngitis, chronic sinusitis, vasomotor rhinitis, deformation of the nasal septum, and other ear, nose, and throat pathology.

By as early as the first year of treatment the number of cases of disease and days of incapacitation in this group of workers decreased by 2-4 times as compared to the year preceding treatment. On the average, disease was not observed at all among 50 percent of the treated persons.

The decline in the number of individual cases of illness in the first year of aerosol therapy is indicative at the Orekhovo-Zuyeva Cotton Combine. The number of cases of angina decreased the most (by 5.5 times); the incidence of acute respiratory diseases, bronchitis, and pneumonia dropped by 2-2.5

times. The number of days of incapacitation associated with ear, nose, and throat morbidity, angina, bronchitis, and pneumonia dropped in the first year of treatment of 525 workers from 10,835 to 3,524 days--that is, by 7,310 days, which made it possible to prevent an economic loss totaling 328,950 rubles.

As a result of the second year of aerosol therapy the number of cases of illness continued to decline, but to a lesser extent than in the first year. In this connection we believe it sufficient to perform aerosol therapy on workers frequently suffering acute respiratory diseases and angina for only 1 year.

Side-effects of the aerosol were observed relatively rarely (in 4-5 percent of the cases), being expressed basically as a slight burning sensation in the nasal cavity. Headaches and insignificant nosebleeds occurred in individual cases. Allergic reactions were not observed. However, before insufflation of the powder we clarified that allergic anamnesis and, if a contraindication existed, the aerosol was not prescribed. Thus the method we developed for aerosol therapy employing dry combined antibacterial and hyposensitizing preparations was tested many times in the course of 6 years at both industrial and agricultural enterprises, indicating its high effectiveness. The latter provides the grounds for recommending introduction of this method into broad medical practice to prevent frequently occurring acute respiratory diseases, angina, bronchitis, and pneumonia.

In addition to the pronounced therapeutic effect, the significant advantages of this method include its simplicity and the minimum time required for its application, making it possible to use aerosol therapy in large collectives.

Our subsequent observations showed that dry aerosols in which oxacillin is replaced by oleandomycin (0.02 gm) and erythromycin (0.025 gm) produced approximately the same effect.

We recommend that the therapeutic and restorative measures be implemented according to the following plan:

1. Revelation of the group of persons often suffering acute respiratory diseases (twice a year and more frequently) and recurrent angina from police morbidity or hospital records.
2. Placement of this group under dispensary surveillance by shop physicians and otorhinolaryngologists.
3. Integrated medical examination of the dispensary group with the participation of an otorhinolaryngologist, a therapist, a stomatologist, and other specialists (as the indications require), and prescription of restorative measures.
4. Intranasal insufflation of dry aerosol (norsulfazol--0.25 gm, oxacillin--0.025 gm, dimedrol--0.025 gm, ephedrine--0.012 gm) by the dispensary group of workers twice in 1 year--in spring and fall--daily for 10 days.

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NEW BOOK DISCUSSES RESPIRATORY PATHOLOGY IN CHEMICAL INDUSTRY

Moscow VESTNIK OTORINOLARINGOLOGII in Russian No 2, 1978 pp 111-112

[Review by E. K. Siyrde of the book "Professional'naya patologiya verkhnikh dykhatel'nykh putey v khimicheskoy promyshlennosti" (Occupational Pathology of Upper Respiratory Passages in Chemical Industry), by I. B. Soldatov, V. A. Danilin, and Yu. V. Mitin, Moscow, Izdatel'stvo Meditsina, 1976, 5,000 copies, 185 pages]

[Text] Publication of a monograph devoted to the problems of chemical industry is quite timely, inasmuch as creation of new toxic compounds and their combination can lead to arising of new clinical forms of occupational pathology.

The authors emphasize that the protective functions of upper respiratory passages suffer considerably when the body is affected by chemical compounds, since the bulk of them enter the body by inhalation. Hence it is clear that one of the most important places in the overall fight against occupational diseases belongs to the otorhinolaryngologist.

Chapter 1 contains a review of nasal physiology, and the authors dwell on the most important protective functions of upper respiratory passages and on the disturbances arising in the body in response to pathological changes in upper respiratory passages.

Chapter 2 reviews hygienic factors having an influence on the state of the upper respiratory passages of workers in chemical industry. These factors include production of synthetic rubber (isoprene rubber from isopentane and isoamylene), as well as the processing of nontoxic isomers of hexachloran (trichlorbenzol) and organophosphoric plasticizers (tricresyl and tricresylenyl phosphates). In addition to chemical factors, ambient air temperature and humidity may have an unfavorable influence on workers.

A complex of chemical compounds in small concentrations, combined with noise and sometimes with disturbance of microclimatic conditions, acts upon the upper respiratory passages and bodies of workers in the chemical production operations noted above, promoting change in the state of upper respiratory passages and an increase in the general morbidity of maintenance personnel.

All of this heightens the responsibility of managers, engineers, and technicians for implementing preventive measures.

Chapter 3 analyzes the state of the upper respiratory passages of workers in chemical production operations. In 10 years, examinations were performed on 3,079 workers in five production operations with different lengths of work careers subjected to the effects of toxic compounds of varying nature. The obtained results were subjected to statistical treatment. It was revealed that catarrhal, subatrophic, and atrophic changes occur most frequently in the upper respiratory passages, and that hypertrophic phenomena occur more rarely. Workers who had not worked for a long time exhibit predominant affliction of the nasal mucous membrane and the pharynx, while those who had worked for a long time reveal afflictions of the pharynx and larynx predominantly. The changes revealed in upper respiratory passages are not specific.

Research conducted to reveal the allergic properties of different groups of chemical compounds showed that some compounds (especially formaldehyde) are allergenic. The sensitization effect depends to a significant extent on the dose of the compound. Skin tests are the principal method used in allergy analysis at industrial enterprises. Chemical compounds settling on a mucous membrane decrease its resistance to microbial infection. The authors established that staphylococcus vegetates most frequently on the skin of workers in chemical production operations, followed by streptococcus and other species of microbes. Microfloral sensitivity was found to be greatest to monomycin, while resistance was found to be greatest to penicillin.

Histological analysis of palatine tonsil tissue and tissue from the lower turbinate bones showed that toxic compounds primarily influence the epithelium of upper respiratory passages. This is followed by reaction of vessels in the subepithelial layer. After atrophy of the epithelial layer, sclerosis of the mucous membrane itself begins.

Chapter 4 describes functional methods for analyzing the upper respiratory passages of workers in chemical industry. The analysis covers olfaction, functions of the ciliary epithelium, the sensitivity of the nasal mucous membrane, the breathing function of the nose, the excretory and assimilatory functions of the nasal mucous membrane, the cellular composition of excreted products, biopotentials, the hydrogen index of nasal excretions, the results of using the luminescent method to assess the state of the mucous membrane, lysozyme activity, the humidity of exhaled air, and rheographical data; determinations were made of the stability of capillaries in the mucous membrane. Data from these analyses differed to a greater or lesser extent from normal indices, which is associated with dystrophic changes in the mucous membrane. An important fact is that these methods permit detection of early manifestations of pathological afflictions and registration of functional disturbances in the absence of visually observable pathology.

Chapter 5 examines the mutual relationship between occupational pathology of upper respiratory passages and changes in other organs and body systems.

Clinical observations and experimental analyses indicate that formaldehyde and dimethyldioxane have toxic properties (for example, they elicit neuroasthenic syndromes and pathological changes in the blood). Among unsaturated hydrocarbons (olephins), ethylene, isobutylene, and isoamylene have intense narcotic properties. Dimethylformaldehyde affects the central nervous system, chlorbenzols and chlorenols affect the nervous and cardiovascular systems, and so on.

Damaging the first protective barrier, which is what the mucous membrane on upper respiratory passages is, with prolonged exposure chemical compounds begin to be assimilated into the blood quickly, and they begin to have a general toxic action upon other protective systems of the body, internal organs, the nervous system, and blood. Consequently one of the ways to decrease overall occupational morbidity at chemical enterprises is to prevent diseases of upper respiratory passages among the workers.

Chapter 6 examines the morphological characteristics of the state of upper respiratory passages in experimental research. In order to study the mechanism behind the influence of products from the isoprene synthetic rubber production operation, or organophosphoric plasticizers, and of chlorinated hydrocarbons on the mucous membrane of upper respiratory passages, the authors performed experimental (cytological and morphological) studies on 650 white rats. The reaction of the mucous membrane to the action of toxic compounds in subacute and chronic experiments can be evaluated as chronic inflammation with a tendency toward atrophy. Results obtained in clinical examinations of workers agree completely with the experimental data.

Chapter 7 is devoted to prevention and treatment of occupational afflictions of upper respiratory passages at chemical enterprises. This chapter notes the principal tasks of occupational pathologists in their joint work with hygienicists, toxicologists, and various clinical specialists, and it emphasizes the important role of enterprise management. Priority is placed on hygienic, engineering, and technical measures. Production processes must be improved so that contact of workers with chemical compounds could be eliminated or reduced. Public health education must be provided with emphasis on mandatory use of personal protective resources, compliance with personal hygiene rules, and control of harmful habits. The authors have published pamphlets for workers for this purpose. The goal of these pamphlets is to acquaint the workers with the influence chemical compounds might have on the human body when industrial safety rules are not complied with adequately.

Therapeutic-preventive measures are important. Sensible occupational selection coupled with mandatory examination of olfactory functions upon acceptance of personnel has great significance. Contraindications to acceptance are chronic rhinitis, deformation of the nasal septums coupled with disturbance of nasal breathing, chronic diseases of accessory nasal sinuses, and impaired olfaction. Repeat examinations, dispensary registration and, when necessary, treatment would be required. Prescription of inhalation procedures must be thought out carefully, with a consideration for the nature of the chemical compounds

in the shops. Moreover the goal of prescribing these procedures--be it therapy or prevention--must be taken into account.

Improvement of working conditions in the shops has led not only to an improvement in the state of the mucous membranes of upper respiratory passages but also to improvement of the indices of general morbidity and elimination of acute occupational intoxications. This chapter is what makes this work practically valuable.

The bibliography contains 268 sources. The monograph is well written, and it is easy and interesting reading.

We could say in conclusion that this monograph, which is a substantial work, will be of great assistance to otorhinolaryngologists as well as to occupational pathologists, hygienicists, and employees of medical-sanitary institutions in their daily work.

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## SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

### FIFTIETH BIRTHDAY OF THE INSTITUTE OF MICROBIOLOGY AND VIROLOGY IMENI ACADEMICIAN D. K. ZABOLOTNYY OF THE UKRAINIAN ACADEMY OF SCIENCES

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 2, 1978 pp 139-147

[Article by V. V. Smirnov, director of the Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy of the Ukrainian Academy of Sciences]

[Text] The Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy, of the Ukrainian Academy of Sciences, will celebrate its 50th birthday on 31 May 1978; this is one of the leading scientific institutions of the Soviet Union. The foundation thereof became an important event in the life not only of the Ukraine, but the entire nation.

Only medical and general microbiology and, to an insignificant extent, soil microbiology developed in prerevolutionary Russia. Early in the 20th century, sanitary bacteriological institutes were founded in some cities of Russia, in particular in the Ukraine, in Kiev, Odessa and Khar'kov, whose main task was sanitary epidemiological work and production of preventive agents. After the October Socialist Revolution, unlimited opportunities were offered for development of microbiological science. The increasing rates of development of industry, large-scale and mechanized agriculture and implementation of routine health-improving measures advanced more and more new and difficult tasks to microbiology, and this stimulated its development.

At the initiative of Academician D. K. Zabolotnyy, the Institute of Microbiology and Epidemiology of the All-Ukrainian Academy of Sciences, in order to concentrate scientific research on the central theoretical problems of microbiology. It was the intention of D. K. Zabolotnyy for this institute to work on theoretical problems of general, agricultural, industrial and medical microbiology that could not be handled by sectorial institutes.

The institute adopted the name of its founder, Daniila Kirillovich Zabolotnyy, academician of the USSR and Ukrainian academies of sciences, member of the VTsIK [All-Russian Central Executive Committee] and VUTsIK [All-Ukrainian Central Executive Committee and president of VUAN [All-Ukrainian Academy of Sciences], by decision of the Sovnarkom [Council of People's Commissars] of the Ukrainian SSR, dated 31 December 1930. In 1944,

the institute was renamed the Institute of Microbiology imeni Academician D. K. Zabolotnyy and in 1963, Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy, UkAS [Ukrainian Academy of Sciences].

This institute, which was small at first, consisting of only two departments, medical microbiology (headed by D. K. Zabolotnyy) and soil microbiology (headed by A. I. Rakitskaya), and was staffed by about 20 members, developed into the leading microbiological institution. Before the Great Patriotic War, it employed 115 people, 48 of whom were scientists and in 1947, 72 and 32, respectively. At the present time, there are 14 scientific departments and 3 unstructured laboratories, employing a large scientific group of 470 people, 165 of whom are scientists, including 3 corresponding members of UkAS, 12 doctors and 132 candidates of sciences.

In addition to the traditional departments of general and soil microbiology, antibiotics, phytopathogenic bacteria, experimental mycology, physiology of fungi, physiology of industrial microorganisms, genetics of microorganisms and biochemistry of microorganisms, several departments were opened in different years: departments of phytopathogenic viruses, reproduction of plant viruses, molecular biology of viruses, technology of biosynthesis, biology of gas-oxidizing microorganisms and radiation microbiology.

In the time of its existence, the institute has done much work dealing with microbiology and virology. In the first few years, research was begun, under the guidance of D. K. Zabolotnyy, on intestinal infections. In the 1930's, one of the main directions of research was shaped at the institute, investigation of theoretical and practical aspects of bacteriophagia and variability of microorganisms (I. Ye. Ruchko, V. G. Drobot'ko, G. M. Frenkel', B. I. Kleyn, F. Ye. Sergiyenko, P. Ye. Vizir', M. L. Nepomnyashchaya and others). A total of 40 different types of bacteriophages of pathogenic bacteria, soil and other microorganisms were obtained, and some of them for the first time (pertussis, brucellosis and others).

Dry dysentery bacteriophage, a combination of dysentery bacteriophage and sulfidine, and staphylophage, which were developed at the institute, were used in prewar and war years. The use of bacteriophages against bacterial wildfire of tobacco lowered the incidence of this disease.

Bacteriophages in the dairy and butyl-acetone industry were first studied in the Ukraine, and this made it possible to develop measures to control these agents that spoiled the products of these industries. Studies of variability of microorganisms were pursued by all departments on the basis of the tenets of dialectical materialism and evolutionary theory of development of the organic world. The institute instigated the convocation of two All-Union conferences on problems of variability of microorganisms and bacteriophagia (1936 and 1953).

One of the major achievements of the institute in prewar years was identification of the etiology of an unknown "NZ" disease of horses, which was very

detrimental to the national economy. It was shown that the disease is caused by toxin of the fungus *Stachybotrys alternans*, which grows on straw. The disease was named "stachybotryotoxicosis." Several members of the institute staff were awarded Orders of the USSR in 1939 for their work on stachybotryotoxicosis (V. G. Drobot'ko, P. Ye. Marusenko, B. Ye. Ayzenman, N. Yu. Kolesnik, D. G. Kudlay, P. D. Yatel', N. M. Pidoplichko and B. O. Kagan). In 1939, the etiology of a second unknown equine disease (Zaporozhskoye NS), induced by toxins of a new fungal species, *Dendrodochium toxicum* (Pidopl. et Bilai) was identified; this fungus also developed on coarse feed. The disease was named "dendrodochiotoxicosis" (N. M. Pidoplichko, V. I. Bilay, F. M. Ponomarenko and B. P. Borisevich). During the war years, the staff of the institute, in collaboration with microbiologists in other institutes, participated in disclosing the etiology of so-called "septic angina," a disease of people that was widespread in Povolzh'ye and the Urals in 1942, as well as in developing preventive measures against it (N. M. Pidoplichko and V. I. Bilay).

During the Great Patriotic War, a significant number of staff members, including the institute director, P. Ye. Marusenko, party organization secretary N. V. Stadnichenko, B. O. Kagan, head of the department of biochemistry of microorganisms, scientific secretary P. Ye. Vizir' and scientists V. S. Rozhdestvenskiy and P. D. Yatel' left for the front. The Institute was evacuated to Ufa, then moved to Moscow (1941-1944) and continued with its scientific work as part of the unified Institute of Zoobiology. At that time, research was pursued on the treatment and prevention of bacterial and fungal diseases.

In the postwar period, much attention was given to restoration of the institute and growth of personnel. The institute was headed by Academician V. G. Drobot'ko of the UkAS, a prominent microbiologist, and thanks to his efforts the institute regained its leading position in microbiology. Work on problems of physiology and biochemistry of microorganisms became the main direction. Among the basic investigations conducted on this topic, there are the comprehensive studies of antibiotics and other biologically active substances of diverse microorganisms, microbiological synthesis of protein, etc. The work dealing with development and introduction to medical practice of the therapeutic agent, microcid, was awarded the USSR State Prize (N. M. Pidoplichko and V. I. Bilay, corresponding members of the UkAs, 1952); the USSR State Prize was awarded as well for studies dealing with development of theoretical bases of microbiological synthesis of protein on petroleum hydrocarbons (Ye. I. Kvasnikov, corresponding member of the UkAs, 1971). State awards (orders and medals), as well as medals and certificates of the USSR and UkSSR BDNKh [exhibitions of achievements of the national economy] and commemorative prizes of the presidium of the UkAS, were bestowed upon many members of the institute staff for other research.

The institute is the coordinating center in the UkSSR for problems of physiology and biochemistry of microorganisms; it is the leading center for problems of phytoncides [bactericide-fungicide-protozoacides from plants], mycology,

destruction of materials by microorganisms, general and industrial microbiology, phytopathogenic bacteria and phytoviruses. The institute conducts research in its departments dealing with three problems: physiology and biochemistry of microorganisms, molecular biology, microbiological synthesis of protein and other products from hydrocarbons.

Studies dealing with soil microorganisms took an important place in the work of the institute; they were begun in the early years of its existence within the framework of the problem of "Correlations between microorganisms and higher plants," and first of all, phenomena of fixing atmospheric nitrogen by microorganisms. Research was conducted in the department of general and soil microbiology under the guidance of N. G. Kholodnyy and D. M. Bogopol'skiy, academicians of UkAS and L. I. Rubenchik, corresponding member of UkAS (1944-1968); since 1968 it has been headed by Ye. I. Andreyuk, doctor of biological sciences. As a result, active strains were obtained of nitrogen-fixing bacteria, phosphobacteria, rhizobia and others, from which nitrobacterin, phosphorobacterin and nitrugin were produced for a long time. Studies dealt with determination of the role in nitrogen fixing of other groups of microorganisms, including oligonitrophils. It was demonstrated that oligonitrophilia is facultative. For the first time, the patterns of distribution of actinomycetes were established in the rhizosphere and soil, and their link with soil productivity was demonstrated; studies were pursued of correlations between bacteria and algae, and their involvement in nitrogen-fixing processes was demonstrated. The department made a considerable contribution to the study of microbiology of corrosion. In the last few years, some important research was conducted to determine the causes of appearance of an aggressive environment during the construction of the Kiev subway. The recommendations proposed by the institute to control corrosion were adopted in subway construction and yielded a large economic effect. The same department conducted a major investigation (V. T. Smaliy, doctor of biological sciences) on the use of specially selected bacteria in the manufacture of biomineral fertilizers, which are now used in the poles'ye [alluvial plain] regions of the Ukraine. More than one million tons of biomineral fertilizers were produced on cultures proposed by the institute.

The department of antibiotics, founded in 1928, was called the department of medical microbiology (headed by D. K. Zabolotnyy). Since 1930, this department was headed for over 30 years by V. G. Drobot'ko, academician of UkAS (also after it was renamed the department of pathogenic microorganisms). The department has had the name of department of antibiotics since 1963 (headed by Prof B. Ye. Ayzenman). At first, research was pursued in the direction of investigation of biological properties of pathogenic microorganisms and their metabolism in order to develop therapeutic agents, bacteriophages, chemotherapeutic agents, as well as investigation of the mechanism of action of chemotherapeutic agents and antibiotics. Subsequently, the main direction of research in this department was development of the theoretical bases of finding antibiotics synthesized by bacteria and higher plants. Problems of antibiotic synthesis were worked on in close correlation to the systematics of these organisms. The search for antibiotics was

conducted to control bacterial, fungal and viral diseases of man, animals and plants, as well as to treat malignant tumors.

New antibiotics were isolated from different bacterial species (phloroglucinol, antibiotic 3187 from representatives of the genus *Pseudomonas* and others), as well as from a number of medicinal plants. Some antibiotics of plant origin-- imanin, novoimanin, salvin, arenarin, product K, product 6--are used in medical practice or are being studied in various branches of medicine. It was determined that they are referable to specific groups of chemicals, or else their chemical structure was identified. Semisynthetic derivatives based on some plant antibiotics are being developed (in collaboration with the Institute of Organic Chemistry, UkAS) and studied, in order to enhance the effectiveness of the original products. Studies are in progress of the mechanism of action of antibiotics on pathogens and their influence on the macroorganism. The fact that there have been six All-Union conferences on the problem of phytoncides, held jointly with Leningrad University, are indicative of recognition of the leading role of this department in research on plant antibiotics.

Extensive research is being conducted at the institute on microscopic fungi. In 1933, the laboratory of mycology was organized (headed by N. M. Pidoplichko, corresponding member of UkAS); in 1936, the department of mycology was founded, and in 1958 it was reorganized into two departments: experimental mycology (headed by N. M. Pidoplichko, corresponding member of UkAS) and physiology of fungi (headed by V. I. Bilay, corresponding member of UkAS). Ecology--systematics and physiology are the main directions of these departments. The fungal flora of various ecological habitats has been well-investigated, and this is of importance to the national economy: phytopathogenic fungi, grain crop fungi, feed, soil and rhizosphere fungi of agricultural plants of the UkSSR. Experimental systematics, approaches and scientific solution of the problem were expressed in the study of fungi of the genus *Fusarium* Lk. In the last few years, there has been successful development of new directions in physiological and ecological studies of fungi under extreme living conditions; studies are in progress of mechanisms of resistance to radiation in dark-colored hyphomycetes, biochemical bases of fungal thermophilia and thermostable enzymes, microflora of fungi that injure material, raw mater, industrial products and installations.

The most effective result was development of a new fungal species, *Penicillium vitale*, and selective cultivation thereof in order to obtain the therapeutic product, microcid, and subsequently the enzymes, glucose oxidase and catalase. These studies elicited a great social and economic effect. Equally important are studies dealing with development of theoretical bases for selective regulation of physiology of fungi that produce the most important, physiologically active substances--enzymes, toxins, amino acids and others.

In the years of operating these two departments, cadres of qualified mycologists in different specialties have been trained.

Comprehensive investigations were conducted at the institute in the field of industrial microbiology. In accordance with the plan of D. K. Zabolotnyy, after organizing the institute and first departments, the department of industrial [technical] microbiology was founded in 1932 (headed by M. N. Nepomyashchaya, 1932-1941). There were two structural laboratories in the department: yeast (headed by N. V. Stadnichenko) and anaerobic (headed by Prof G. M. Frenkel'). In 1945, the department was renamed department of microbiology of fermentation processes, then department of physiology of industrial microorganisms (headed by M. L. Nepomnyashchaya, Prof G. M. Frenkel' and, since 1960, by Ye. I. Kvasnikov, corresponding member of UkAS).

During the prewar period, studies were pursued of the slime pathogens of sweetened juices [sugar juices] and bread, and methods were developed to control them. Subsequently, the department worked on problems of bacteriophagia, theoretical aspects of anaerobiosis, etc.

In the last few years, a comprehensive cycle of works was conducted on lactobacteria (methods of research, ecology, physiology, biochemistry) and practical significance thereof. Recommendations were prepared and introduced, with a significant economic effect, on regulation of vital functions of lactobacteria in several branches of the national economy (preservation of feed, alcohol industry, viniculture). The basic direction is research on biosynthesis of protein from petroleum hydrocarbons. A new heat-tolerant strain, *Candida tropicalis* K-41, was bred and introduced in the production of protein-vitamin concentration (BVK). Studies have been pursued of hydrocarbon-assimilating species of other microorganisms, noncardia, corynebacteria and bacteria. Strains of micrococcus have been bred that have high biosynthetic activity when cultivated on n-alkanes (amino acids, vitamins). A new strain has been patented in several foreign countries.

The department of phytopathogenic bacteria was founded in 1934 (it was headed by Prof K. I. Bel'tyukova for 33 years and by R. I. Gvozdyak, doctor of biological sciences, since 1970).

Studies were pursued for detection and investigation of bacteriosis in the Ukraine. There were studies of numerous pathogens of bacterial diseases of the principal agricultural crops, fruit, trees, and recommendations were offered for the control thereof.

A correlation was demonstrated between some biological properties of pathogens, their virulence and pathogenicity. New tests and methodological procedures were introduced for isolation and investigation of phytopathogenic bacteria. It was shown that, in plants, the infectious process is associated with a change in intensity of respiration, activity of enzymes of protein and nitrogen metabolism.

Various bacteriophages were isolated and studied; some of them have been recommended for practical use. A thermochemical method was developed for the

control of gummosis in cotton plants, as well as a method of using the antibiotic arenarin and analogues of the natural antibiotic, allicin, for the control of bacterial canker of tomatoes and other diseases.

The role of this department in research on phytopathogenic bacteria is recognized by the country's leading specialists, as also indicated by the fact that the department convoked two All-Union conferences.

The department of biochemistry of microorganisms was founded a few years before the war (headed by B.O. Kagan and, after the war, by Prof Ye. M. Koldayev).

The department of biochemistry of microorganisms was founded as a permanent functional structural entity at the institute in 1951 (headed by Prof Ye. Ya. Rashba and then by I. Ya. Zakharova, doctor of biological sciences). At first, research was pursued on biochemical properties of atypical forms of bacteria.

The use of the latest methods of chemical, physicochemical and immunochemical analysis, as well as development of the L-17 homogenizer to break down microorganisms, made it possible to conduct the most refined studies of acellular extracts and various structural units of the cell. Some aspects were identified of the mechanism of biological fixing of molecular nitrogen in azotobacter, and involvement of different enzymes (hydrogenases, nitrogenase complex and several enzymes of ATP synthesis) in this process.

The structure of the O-specific side chain of lipopolysaccharides of two strains of enteropathogenic *E. coli* 020 bacteria was identified. Some differences were demonstrated in the composition of lipopolysaccharides of the two strains, which determine the presence of different O-antigen factors in them. The lipopolysaccharides of phenotypically similar *Bact. faecalis* alcaligenes and enterobacterial mutants contain lipophilic sugar (first identified in this department), which has immunodominant properties; this served as proof of phylogenetic identity of the cultures studied.

Investigation of various representatives of oligonitrophils revealed that their extracellular slime is pure polysaccharide. It was established that there is a certain link between molecular ratios of monosaccharides in extracellular polysaccharides of oligonitrophils and the systematic place of this group of microorganisms.

Strains of microorganisms have been discovered, which have considerable galactose oxidase and mannanase activity.

A proteolytic set of enzymes produced by the radiation mutant of *Bac. mesentericus* 316m has been isolated and is being studied. This enzyme complex has caseinolytic, elastolytic, hemoglobinglytic and fibrinolytic activity. A fraction has been isolated from the enzyme preparation, that has only elastolytic activity.



In-depth studies have been conducted in the field of genetics of microorganisms and radiation mutagenesis.

Studies of variability of bacteria, started at the institute long ago, obtained further development in the department of variability of microorganisms opened in 1955 (headed by Prof P. Ye. Vizir'), which was renamed in 1964 as the department of genetics of microorganisms (headed by Prof P. Ye. Vizir', and B. P. Matselyukh, candidate of biological sciences).

As a result of genetic analysis of actinomycetes (B. P. Matselyukh), the circular map was plotted of the genome of *Streptomyces olivaceus* V KX, on which over 40 genetic loci were charted. For the first time, it was shown that there is bidirectional replication of the chromosome of actinomycetes and the time intervals were determined on the genetic map of replication of DNA of several genes; for the first time, the superhelical plasmid was isolated, which determines synthesis of antibiotic and resistance to it, and which also plays a role in genetic exchange in actinomycetes. A hypothesis has been expounded for the mechanism of genetic recombination in actinomycetes.

A genetic method was developed for breeding bacteria, with which a more active and slime-free strain was obtained of the industrial producer of polymyxin B.

The department of radiation microbiology was founded in 1961 (headed by A. M. Pasechnik, candidate of biological sciences). Its main direction of research is the study of the mutagenic effects of ionizing radiation on bacteria that produce physiologically active substances. A comparative study was made of the mutagenic effects of ultraviolet rays, gamma radiation and fast neutrons of the VVR-M experimental nuclear reactor on the example of industrial strains of microorganisms of the genera *Bacillus* and *Clostridium*, and it was demonstrated that fast neutrons are highly effective in inducing variants with altered enzymatic activity. As a result of delivery of low intensity doses and stepped selection, for the first time types A and B of *Cl. perfringens* strains were obtained by experimental breeding, which have higher biosynthetic activity and which are promising for the production of therapeutic products, as well as strains that are deficient in synthesis of some enzymes, that are suitable for studying the routes of biosynthesis thereof. A strain of the producer of proteinases, *Bac. mesentericus* 316m, was obtained that is 26% better than the original strain with regard to level of gelatinase activity. The bred strain has been introduced in the production of photosensitive materials, where it is used in the technological process of film regeneration.

Last to be organized were the departments of technology of biosynthesis, chemistry of raw material and biology of gas-oxidizing microorganisms.

Investigations dealing with microbial synthesis of protein from petroleum hydrocarbons, agricultural waste and methanol are the main directions of work in the department of technology of biosynthesis (headed by V. F. Semenov,



candidate of engineering sciences). Technological regulations and equipment have been developed for processes of biosynthesis, and industrial trials have been made. In the last 5 years, the economic effect of introducing the projects of the department in industry constituted several million rubles.

Important research conducted by V. Ya. Masumyan dealt with microbial synthesis of feed protein; he studied the chemistry of raw material and of microorganisms cultivated on n-alkanes.

The department of biology of gas-oxidizing microorganisms was founded in 1968 (headed by Yu. R. Malashenko, candidate of biological sciences). In a relatively short period of time, much work was done and there was demonstration of the distribution of methane-oxidizing bacteria in various ecological niches; new thermophil gas-oxidizing microorganisms have been described. Mathematical models have been constructed of growth of microorganisms on natural gas and methanol. It was established that the group of obligate methylotrophs is unique, which warranted classification thereof in a separate family. There has been elaboration of principles of classifying genera, species, and keys have been provided for identification thereof. New species were described. For the first time, a new type of raw material, methanol, was proposed in the USSR for synthesis of BVK; active yeast cultures have been bred, and a method was developed for producing BVK on the basis of methyl alcohol.

In the department of natural antineoplastic agents (headed by D. G. Zatula, corresponding member), which functioned from 1970 to 1977, studies were pursued of microbiological aspects of oncology. A strain of *Bac. megaterium*, which was discovered and which has antigenic affinity for tumor cells, was used for selection of antineoplastic substances. Studies have been pursued of antineoplastic properties of plant antibiotics and plant extracts, as well as culture fluids of microorganisms.

There has been research on the microfloral composition of the gastrointestinal tract of patients with malignant tumors. A clinical study of plant product 6 was pursued in collaboration with the department of antibiotics.

The work done at the institute dealing with viruses of plants, animals, algae, fungi and bacteria is very important.

Comprehensive investigation of phytopathogenic viruses began in 1960, when the department of plant viruses was founded (headed by S. N. Moskovets, corresponding member of UkAS). As a result of the research done in this department, the phytovirological situation in the Ukraine was determined: presence and incidence of the main viral diseases of the principal agricultural crops and their economic significance. Methods were developed for diagnostics and identification of most of the studied diseases and their pathogens; the ways and means of their transmission, dissemination and circulation in nature were identified. Recommendations dealing with the

control of viral diseases of potatoes, tobacco, tomatoes, sugar beets, hops, leguminous and other crops were prepared and submitted to the Ukrainian Ministry of Agriculture.

Subsequently, independent departments and unstructured laboratories were opened on the basis of the department of plant viruses.

In the department of phytopathogenic viruses (headed by A. D. Bobyr', doctor of biological sciences), research was pursued, which resulted in demonstration of a considerable number of various substances capable of depressing several phytopathogenic viruses. The general principles were elaborated for searching and selecting, for practical purposes, the most effective inhibitors; the ways and means have been outlined for extensive use thereof in the control of viral diseases of cultivated crops.

More than 20 antiviral agents were obtained from the metabolic products of yeast of the genera *Candida* and *Saccharomyces*; their polysaccharide nature was established, and studies were made of the physicochemical properties and biological activity thereof with reference to a number of phytopathogenic viruses.

Studies are in progress of the nature of genetically determined virus resistance of plants. Methods have been developed for cultivation, isolation and purification of viruses that strike fungi of the genus *Penicillium*; their physicochemical, biological, antigenic, inhibiting and inducing properties have been studied.

The department of reproduction of plant viruses (headed by V. G. Krayev, candidate of biological sciences) is concerned with research in the field of interaction between plant viruses and cells. For the first time, it was established that potato X and Y viruses can multiply in the same cell. Studies have been made of the components of the virus of red clover mottling; it was established that the virions of each component contain two proteins differing in molecular weight. Several new viruses of blue-green algae were isolated and studied in the unstructured laboratory of algal viruses headed by V. A. Goryushin, candidate of biological sciences: cyanophage AM-1, which lyses unicellular blue-green algae; cyanophages A-1, A-2 and N-2; local strains of cyanophage LPP-1 and cyanophage LPP-3, which lyse filamented blue-green algae. The phenomenon of lysogeny was discovered in unicellular blue-green algae. In addition to viruses of blue-green algae, a virus that strikes the green algae, *Chlorella pyrenoidosa*, was isolated in this laboratory.

In the departments dealing with plant viruses and specialized unstructured laboratories, a broad front of research has been deployed on morphology and fine structure, physicochemical, antigenic and other properties of viruses of plants, blue-green algae and microscopic fungi, their relations to host cells, localization, reproduction, etc.

From 1963 to 1968, there were the following departments at the institute: department of animal viruses (headed by Academician S. N. Gershenzon of the UkAS), department of chemistry of nucleic acids and viral carbohydrates (headed by V. P. Chernetskiy, doctor of chemical sciences), which subsequently merged into the Institute of Molecular Biology and Genetics, UkAS.

In 1963, the department of physical methods of studying viruses was organized at the institute; in 1964 it was renamed the department of viral biophysics (department head: Ya. G. Kishko, doctor of biological sciences), and since 1977 it has been called the department of molecular biology of viruses (acting department head: N. S. Dyachenko, doctor of biological sciences).

The main direction of this department was investigation of the fine structure of viruses and molecular mechanisms of their reproduction. Studies have been made of the ultrastructure of several viruses. The parameters of whole vibriions, elements of the capsid and isolated nucleic acids have been determined. It was demonstrated that *Ps. vignae* bacteria have exceptional polysogeny, which is induced by two different temperate phages (Ya. G. Kishko).

Substantial differences were demonstrated in physicochemical properties and antigenic structure of adenoviruses; a new antigen of adenovirus and virus-neutralizing activity of antibodies to type 1 hexon were discovered. For the first time, it was determined that accumulation of DNA, RNA and protein is limited to the early stages of formation of inclusions. New data have been obtained on the effects of DNA of genome viruses (adenoviruses, some phages) and their components on the immunological system of experimental animals (N. S. Dyachenko).

The staff of the institute has always tried to concentrate all efforts and material resources on the study of the most pressing problems of modern microbiological and virological science, and to use the theoretical results obtained as the basis in solving important practical problems of building of communism. The institute maintains solid and broadening ties with industry along the lines of medicine, microbiological and fermentation industry, and agriculture. The institute conducts its scientific research and scientific organizational work in conjunction with 68 scientific institutions and enterprises, with reference to various problems, as well as on the basis of socialist competition with the nation's scientific research institutions and enterprises. The institute is working on five projects together with scientific institutions of GDR and CSSR.

The scientists at the Institute of Microbiology and Virology imeni Academician D. K. Zabolotnyy, UkAS, have always attributed and continue to attribute much importance to the training of scientific personnel and microbiological specialists for scientific research institutes, medical, agricultural and industrial institutions.

For many years, the institute has been the "factory" for scientific cadres of microbiologists and virologists, for both our country and a number of

other countries--Czechoslovakia, Bulgaria, Poland, Afghanistan, India and Syria.

Republic-level and All-Union seminars, symposiums, conferences of young specialists dealing with pressing problems of modern microbiology and virology are held regularly at the institute.

Since 1934, the institute has been publishing MIKROBIOLOGICHESKIY ZHURNAL [Microbiological Journal], monographs, collections on different topics and methodological works. In the last 5 years alone, it has published 29 monographs and 13 collections of works; it has received 30 author certificates, 7 diplomas and 14 medals of the USSR VDNKh and UkSSR; 4 people have been awarded the commemorative D. K. Zabolotnyy Prize.

In celebrating the 50th anniversary of the institute, its staff is directing all work toward fulfilling the decisions of the 25th CPSU Congress and 25th Congress of the Communist Party of the Ukraine, toward implementing the new tasks of building communism.

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## SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

### PLENUM OF THE PROBLEM COMMITTEE ON THE SCIENTIFIC BASIS OF HYGIENE AND TOXICOLOGY OF PESTICIDES, POLYMERS AND PLASTICS

Moscow GIGIYENA I SANITARIYA in Russian No 3, 1978 pp 114-115.

[Article by P. E. Sova, candidate of medical science]

[Text] At the plenum of the Problem Committee a paper was read by Academician of the USSR Academy of Medicine L. I. Medved' on "The main results of the investigation of the problem in 1976 and the next steps to be taken". In 1976, 346 themes were chosen for investigation at 53 research institutes including 278 themes on the hygienic use of pesticides, their toxicology and clinical treatment of people poisoned by them, as well as 68 themes on hygienic evaluation of polymeric materials. In 1976 the toxic properties of more than 250 chemical materials were studied, and the bases for hygiene standards concerning 43 pesticides were given. New developments were started such as the hygienic evaluation of plant-growth regulators and the hygienic application of mineral fertilizers. The health condition of the population was investigated in areas where pesticides were used in different amounts. Methods were developed for diagnosing prenosologic forms of pathology of chemical etiology with the goal of finding ways to halt their development, which is considered the main approach towards the solution of the problem. Research on various hygienic aspects of the use of polymeric materials was conducted by 14 research institutes. New polymeric materials and methods of applying them in the food industry were evaluated hygienically. The hygienic evaluation research of polymeric materials used in agricultural construction and construction of medical and health care centers was completed. Polymeric materials were evaluated as a new source of pollution of potable water, and preventive measures were developed. Research of polymeric materials as a possible source of allergies was widened. In 1976 seven monographs devoted to the problem were published. The number of cases of practical application of research results has steadily increased. Thus, if in 1975 215 various research materials were ready for practical application, then in 1976 such materials numbered 291; that is, a 35 percent increase. At the same time, in some institutes insufficient attention is given to practical implementation of scientific achievements in the system of health care and the national economy.

A vast scope of research took place in the area of international cooperation. For example, four research institutes are conducting research of environmental hygiene according to the intergovernment agreement between the USSR and the United States.

At the plenary session reports were presented by E. A. Melnikova "On the modern state and prospects of research in the field of biological pesticides", by Y. A. Kuchak on the "Organization of the system of state control of traces of pesticides remaining in the environment using computerized technology", and by V. I. Martynenko "On the nomenclature of pesticides and the prospect of their use in national agriculture until 1990".

Reports were presented on scientific research conducted in accordance with the plan for the national economy and itemized scientific plans for 1976, as well as practical implementation of research results in national health care and the economy.

At the Sessions of the 4 sections (hygiene of the application of pesticides, hygiene of the application of polymeric materials, experimental pathology of chemical etiology and clinical pathology of chemical etiology) recommendations for completing the itemized scientific plan for 1978 were adopted.

In the plenum's decree of the institutes conducting planned research were urged to increase the effectiveness of the practical implementation of research results. It was decided to take measures for the expansion of the scope of research devoted to protecting the soil and reservoirs from pollution by pesticides and mineral fertilizers; to promoting the hygienic application of biological pesticides; to developing methods of determining components of polymeric materials in food stuffs; to studying remote consequences of the action of chemicals; to studying the combined, complex and composite affect of chemicals, physical factors and biological pesticides; to developing methods of diagnosing prenosologic forms of pathology of chemical etiology.

The participants of the plenary session approved recommendations for compiling a itemized research plan and a draft plan for 1978.

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## SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

### SECOND ALL-UNION SYMPOSIUM ON SOMATIC POLYPLOIDY

Moscow TSITOLOGIYA in Russian No 4, 1978 pp 482-484

[Article by Yu. A. Magakyan]

[Text] On 9-12 November 1977, in Yerevan, was held the Second All-Union Symposium on Somatic Polyploidy\*, the organizers of which, as of the First, were the Scientific Council for Problems of Cytology of the Academy of Sciences (AS) USSR, the Scientific Council for the Problem "Regularities of the Individual Development of Animals and the Control of Processes of Ontogenesis" of the AS USSR and the Institute of Zoology of the AS Armenian SSR.

Opening the work of the symposium, the director of the Institute of Zoology of the AS Armenian SSR, S. O. Movsesyan, emphasized the urgency of the tasks facing the biologists of our country in the area of investigations of somatic polyploidy. In the report of V. Ya. Brodskiy (Institute of the Biology of Development of the AS USSR, Moscow) the results of investigations of that phenomenon during the period since the First Symposium were summed up. As a result of those investigations the biological significance of the polyploidization of somatic cells was clarified, the widespread character of the phenomenon was established, and new proofs were given of the special path of development of polyploid cell populations and their role in processes of growth and development of the organism. The reporter characterized the main tendencies of recent work and emphasized the great prospects of investigation of the interrelationship between cell proliferation and differentiation, the differentiating importance of the polyploidization of cells and the mutual influences of different cell functions. O.G. Stroyeva and I. G. Panova, V. M. Faktor and I. V. Uryvayeva (Institute of the Biology of Development of the AS USSR, Moscow) and V. F. Malyutin et al. (Institute of the Biology of the Sea, Far Eastern Scientific Center, AS USSR, Vladivostok, and the Institute of the Biology of Development of the AS USSR, Moscow) examined the mechanisms of polyploidization of cells of different populations and discussed

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\*The First Symposium was also held in Yerevan, in 1971; see TSITOLOGIYA, 14, 5: 680, 1972.

hypotheses of the reasons for the formation of polyploid cells, in particular the hypothesis that the level of proliferative activity and polyploidization of cells in a population is regulated by factors of the general development of the population (O. G. Stroyeva and I. G. Panova). Ye. V. Zybina et al (Institute of Cytology of the AS USSR, Leningrad) reported on an interesting phenomenon of genome fragmentation of giant nuclei of the trophoblast after achieving high degrees of ploidy. M. V. Kudryavtseva et al (Institute of Cytology of the AS USSR, Leningrad), on the example of glycogen accumulation in liver cells, showed that the functional possibilities of the cells grow correspondingly to the increase of ploidy. D. P. Museridze and I. K. Svanidze (Institute of Physiology of the AS Georgian SSR, Tbilisi) on the model system "neuron-glia" in a culture established that the quantity of DNA in the nuclei increases from the moment of stabilization of intercellular bonds in the system.

At the second session M. K. Pavlova and V. I. Malyuk (Institute of Hydrobiology of the AS Ukrainian SSR, Kiev) showed the possibility in principle of controlling the process of polyploidization of plant cells in a culture. L. I. Usenko (Technological Institute of Antibiotics and Enzymes, Leningrad) presented data on the presence in the hyphas of fungi of micronuclei with condensed chromatin and vegetative nuclei of polygenome origin. The former are genetically inactive, but the latter participate in the formation of heteroploid nuclei which are observed in differentiating hyphas. V. P. Bannikova and S. P. Shpilevaya (Institute of Botany of the AS Ukrainian SSR, Kiev) established that in green algae during the differentiation of male gametes the number of polyploid cells increases and the chromatin is progressively condensed. L. V. Gerbil'skiy et al (Medical Institute, Dnepropetrovsk) showed that during the metamorphosis of tadpoles DNA synthesis increases in the cells of various organs during a sharp reduction of mitotic activity. In the opinion of the authors this testifies to DNA hyperreplication at that time. V. A. Vorob'yev (Institute of the Biology of the Sea, Far Eastern Scientific Center of the AS USSR, Vladivostok) reported that the differentiation of the flagellar epithelium of the stomach of asterids correlates directly with the polyploidization of nuclei, and the latter leads to intensification of the motor function of the cells.

In the reports of Yu. A. Magakyan, and also of Ye. M. Karalova and M. G. Khachatryan (Institute of Zoology of the AS Armenian SSR, Yerevan), which were heard at the third session, on the basis of data obtained in studying developing populations of cells in embryogenesis, early postnatal ontogenesis and oogenesis of different species of animals, the concept proposed earlier by Yu. A. Magakyan was developed, according to which DNA hyperreplication is a necessary condition for the accomplishment of processes of differentiation and specialization of cell populations. The authors think that the main factor inducing cells to change to complete or partial endoreplication is regulation of the processes of differentiation and proliferation on the population level, and each cell has potentially the possibility of "selecting", depending on the situation, a given way of increasing the matrix material. In the reports of Z. P. Zaychikova, A. K. Vinogradov et al (Institute of Cytology of the AS USSR, Leningrad), G. I. Bakhtadze (Institute of



Zoology of the AS Georgian SSR, Tbilisi), and A. G. Istomina and L. V. Vysotskaya (Institute of Cytology and Genetics, Siberian Department, AS USSR) questions of the polyploidization of the cells of gonads of various insects were examined, as well as their behavior in the processes of oogenesis and spermatogenesis, methods of endoreplication of the genome, the specific state of the chromosomes in polyploid cells, etc. G. I. Bakhtadze expressed the hypothesis that polyploidization, at least of some types of cells, can be regarded as a manifestation of a certain type of differentiation, the final stage of which is cells with a specific structure of the chromosomes.

G. D. Tumanishvili (Institute of Experimental Morphology of the AS Georgian SSR, Tbilisi) at the fourth session examined questions connected with excess (labile) DNA. On the example of hepatocytes of chicken embryos it was shown that in their nuclei the DNA of heterochromatin can be diminished and rapidly restored. In the latter case excess DNA forms as a result of one of the forms of its hyperreplication. In the author's opinion, in the cells there is a mechanism which triggers the synthesis of additional DNA when it becomes necessary to increase its quantity. In the reports of G. G. Gasparyan, G. G. Zakaryan and L. O. Abroyan et al (Institute of Zoology of the AS Armenian SSR, Yerevan), questions relating to the dependences between the processes of proliferation and differentiation of cells were examined. On model systems in a culture it was shown that during stimulation of cellular multiplication in stationary cultures a portion of the cells can leave the cycle in the premitotic phase without passing through mitosis; this testifies to the presence of two stages in the stimulation of proliferation: stimulation of DNA synthesis and activation of the mechanism of fission itself. It has also been established that the cells, in leaving the cycle in the presynthetic phase, can synthesize protein in larger quantities than is observed in proliferating cultures, while completely preserving in that case potency for proliferation. T. V. Dmitrevskaya (Borkhsenius) et al (Institute of Cytology of the AS USSR, Leningrad, and Institute of Epidemiology and Microbiology of the Academy of Medical Sciences USSR, Moscow) and T. A. Ogloblina (Moscow University) established that polyploid cells in a culture are incapable of forming colonies but diploid cells of Rauscher leukemia multiply and are capable of self-maintenance of strains. Some increase of the amount of DNA in an epidemic control vaccine culture (SPEV) is connected, not with additional DNA replication, but with passage of cells through the cycle.

K. G. Gazaryan (Moscow University) and Ye. G. Korvin-Pavlovskaya et al (Moscow University, Institute of Zoology of the AS Armenian SSR, Yerevan, and Institute of Atomic Energy, Moscow) at the fifth session presented material obtained by complex cytochemical and morphological analysis of cells of the erythroid series under conditions of anemia, during which a method of cytodifferentiation latent in the normal, "reserve erythropoiesis", is induced. In that case the processes of differentiation and specialization are intensified; they are completely accomplished in cells which have left the cycle in the postreplicative period, that is, those containing a doubled quantity of DNA. In that case the cells, having passed along the entire path of cytodifferentiation, including hemoglobin accumulation, divide, forming a diploid population of mature erythrocytes. The authors develop a concept according

to which the processes of differentiation proceed mainly in the postreplicative period of the cycle. This probably also occurs in the process of differentiation of embryonal tissues, but can be detected only under conditions of dissociation during processes of proliferation and differentiation which are created during anemia. Observed together with that under conditions of anemia is a second path of intensification of hemoglobin accumulation in the process of cell specialization, during which those processes are accomplished on the basis of the hyperdiploid content of DNA in the nuclei of erythroid cells. A. N. Mosolov et al (Medical Institute and Institute of Clinical and Experimental Medicine, Academy of Medical Sciences USSR, Novosibirsk) showed that the organization of chromatin in high-ploid nuclei is characterized by a well-expressed tendency toward toroidal compactization. Z. A. Rabinina (Institute of Morphology of Man of the Academy of Medical Sciences USSR, Moscow) thinks that in the course of liver growth and differentiation the degree of participation of polyploid cells in those processes depends on the passage of the animals through "critical" periods of development. G. Ye. Onishchenko and Yu. S. Chentsov (Moscow University) established that in those cells, the polyploidization of which proceeds through mitosis, a distinct correlation is observed between the quantity of centrioles and the number of chromosome sets. G. S. Kvinikhidze (Institute of Zoology of the AS USSR, Tbilisi) reported on cytochemical features and the establishment of the ultrastructure of cells of the retina in the process of differentiation in connection with the dynamics of the DNA content in their nuclei.

The sixth session was devoted to methodical questions of quantitative cytochemistry. In the reports of L. S. Agroskin, G. V. Panayan and G. S. Plagov, I. Ya. Barskiy and A. V. Yakubenas (Optical Institute, Leningrad) original designs of new equipment and methods of using it, methods of estimating instrument errors, etc, were presented. M. V. Arkhinov et al (Institute of Cytology of the AS USSR and the Agrophysical Institute of VASKhNIL, Leningrad), G. S. Lebedeva and A. V. Diment (Institute of Cytology of the AS USSR, Leningrad) and N. V. Kozlova (Institute of Experimental Morphology of the AS Georgian SSR) presented for discussion methodical procedures for quantitative and qualitative investigation of the state of chromatin.

In the last session V. N. Yarygin and I. M. Filichkina (Second Moscow Medical Institute) reported on morphological and cytochemical features of mononuclear and binuclear neurocytes of the sympathetic ganglia of the frog. Substantially more RNA is synthesized in the binuclear neurocyte than in the mononuclear: binuclear cells have a greater mass than cytoplasm. The reports of G. I. Moskovkina (Institute of the Biology of Development of the AS USSR, Moscow), L. Ye. Nemirovskiy et al (Second Moscow Medical Institute), N. N. Belyayeva et al (Institute of General and Communal Hygiene of the Academy of Medical Sciences USSR and Institute of the Biology of Development of the AS USSR, Moscow), Z. F. Veselovskaya and Ye. Ya. Pankov (Medical Institute, Khar'kov), V. F. Kozlova et al (Institute of Problems of Cryobiology and Cryomedicine of the AS Ukrainian SSR, Khar'kov), G. G. Samsonidze and K. N. Barabadze (Institute of Experimental Morphology of the AS Georgian SSR, Tbilisi), and E. I. Gasparyan and S. P. Dzhandzhapanyan (Institute of General Hygiene and Occupational Diseases of the Ministry of Public Health Armenian

SSR, Yerevan) were devoted to establishment of the dependences between the DNA content in the nuclei of cells of different organs and tissues and the development of given pathological processes induced by active substances. It has been shown that hyperthyroidism inhibits the proliferation of cells of the central nervous system and increases the length of the replicative and postreplicative periods, the alkylating agent dipine exerts a stable modifying effect on the process of age polyploidization of hepatocytes, and vitamin A increases the mitotic activity and leads to the accumulation in the tissue of cells with a low content of nucleic acids. On the whole it has been shown that chemical factors relating to different classes of compounds can lead to both elevation of the level of the DNA content in the nuclei of cells and activation of proliferation. Future investigations in that direction will contribute to the determination of the mechanisms of the pathology and the role in them of cells with a higher DNA content.

At the end of the symposium a general discussion was held and the results of its work were summed up. The speakers in the discussion, G. D. Tumanishvili, O. G. Stroyeva, V. N. Yarygin, V. I. Nozdrin, Yu. A. Magakyan, K. G. Gazaryan and V. Ya. Brodskiy, noted the high level of the work presented at the symposium, their timeliness and the large share of participation of young investigators. There was very wide discussion of questions of the interdependence between processes of differentiation and specialization of cells and hyperreplication of DNA in their nuclei and questions of the dependence between those processes and the passage of cells through different phases of the mitotic cycle. Those questions, and also questions relating to the mechanisms of formation of polyploid cells and the role of the latter in the development of cell populations, can be considered very promising for future investigations. The development and investigation of corresponding model systems can play a large role in their solution.

The speakers highly rated the work of the organizing committee of the symposium and noted the business-like character of all the sessions, thanks to the purposefully compiled program and the invitation to participate in the symposium of investigators actively working in that area.

Taking into consideration the work of the First and Second symposiums, the participants decided that the next symposium on somatic polyploidy would be held in Yerevan. It is proposed to hold the Third Symposium in 3 or 4 years.

Thanks were expressed to the organizers of the symposium in the name of the scientific councils for problems of cytology and the problem "Regularities of the Individual Development of Animals and Control of Processes of Ontogenesis" of the AS USSR.

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